DER EINFLUSS VON MATERNALEM PSYCHOSOZIALEN STRESS AUF DIE FETALE VASKULÄRE ENTWICKLUNG

- im Modell des fetalen Schafes-

Dissertation

zur Erlangung des akademischen Grades

„doctor rerum naturalium“ (Dr. rer. nat.)

vorgelegt dem Rat der Medizinischen Fakultät
der Friedrich-Schiller-Universität Jena

von M. Sc. Molecular Medicine Julia Josephine Müller

geboren am 11.08.1989 in Nordhausen

Gutachter

1. Prof. Dr. med. Otto W. Witte

Hans-Berger-Klinik für Neurologie, Universitätsklinikum Jena

2. Prof. Dr. Med. Matthias Schwab

Hans-Berger-Klinik für Neurologie, Universitätsklinikum Jena

3. PD Dr. med. Martin G. Frasch

Dept. Obstetrics & Gynecology, Health Sciences Building, Seattle, Washington

Tag der öffentlichen Verteidigung: 07.07.2020

Inhaltsverzeichnis

Abkürzungsverzeichnis

11β-HSD2	11β-Hydroxysteroid-Dehydrogenase2
α_{1A}	Adrenorezeptor α1A
β_2	Adrenorezeptor β2
ACh	Acetylcholin
AChRM2	muskarinischer Acetylcholin-Rezeptor 2
AChRM3	muskarinischer Acetylcholin-Rezeptor 3
ACTH	Adreno-Corticotropin
Ca^{2+}	Kalzium
COX-2	Cyclooxygenase 2
CRH	Corticotropin-Releasing-Hormons
eNOS	endotheliale NO-Synthase
EP2	Prostaglandin-E2-Rezeptor
ET-1	Endothelin-1
ET_A	Endothelin-1-Rezeptor A
ET_B	Endothelin-1-Rezeptor B
HPA-Achse	Hypothalamus-Hypophysen-Nebennieren-Achse
K^+	Kalium
MHC-B	fetale *smooth muscle myosin heavy chain* isoform
MPS	maternaler psychosozialer Stress
NA	Noradrenalin
NO	Stickstoffmonoxid
PGE2	Prostaglandin E2
RAAS	Renin-Angiotensin-Aldosteron-System
SM2	adulte *smooth muscle myosin heavy chain* isoform

SNP	Sodium Nitroprussid
WHO	World Health Organisation

Abbildungsverzeichnis

1 Summary

Maternal psychic stress (psychosocial stress (MPS) in animals) during pregnancy appears to be an essential factor in the predisposition to cardiovascular disease later in life. Epidemiological data show that the blood pressure of children during the pregnancy of stressed mothers is elevated. Little is known about the underlying mechanisms.

This makes the study of the mechanisms of stress-related programming of the cardiovascular system under increasing stress in society an important task of our time. In this work, the functional and structural development of important fetal vessels involved in blood pressure regulation and the modulation of maturation by chronic MPS depending on the time of pregnancy were examined for the first time in the most important animal model of the human fetal period, the fetal sheep. The functional maturation of vasodilatation and vasoconstriction mediating vascular mediators was investigated by *small vessel wire myography* on distal renal interlobular arterioles and mesenteric resistance vessels. The structural maturation of the blood vessels was determined by histology and the expression of enzymes and receptors involved in vasoconstriction and vasodilation was immunohistochemically investigated.

We were able to determine that the structural maturation of both vessel types was already completed at the beginning of the 3rd trimenon, but that mesenteric blood vessels matured functionally in the last trimenon. This maturation is characterized by an increase in vasoreagibility due to increased contractility of the vessels to K^+, endothelin-1 and noradrenaline and increased vasodilatation to acetylcholine and prostaglandin-E2. Overall, functional maturation was not accompanied by an increase in the expression of vasoreactive receptors (AChRM2, AChRM2, EP2, ETA, ETB, α1A, β2) or enzymes (eNOS, COX-2).

The influence of MPS on vascular maturation depends on the time of stress exposition. Irrespective of the time of stress measurement, MPS led to a delayed maturation of the vascular muscle cells in mesenteric but not renal vessels, indicated by a change in myosin composition, but was not reflected in a change in vessel diameter ("media cross-sectional area", "media-to-lumen ratio").

Functionally, MPS led to an increase in vasoconstriction (response to endothlin-1) in the mesenteric vessels and increased vasodilatation (in response to acetylcholine) in the renal vessels at the end of the second trimenon. These effects were replaced by decreased mesenteric noradrenaline mediated vasoconstriction and increased renal vasodilatation (NO, prostaglandin-E2) 30 days after MPS, i.e. at the end of the 3rd trimenon, possibly to compensate for the initial stress-induced increased vasoconstriction. MPS in the 3rd trimenon did not affect vasoconstriction, but increased vasodilatation (in response to acetylcholine)

in the mesenteric vessels and decreased NO-mediated response of renal vessels. The functional stress effects were not accompanied by a change in receptor or enzyme expression independent of gestational age, suggesting a modification of intracellular signalling pathways by MPS. Therefore, the investigation of intracellular signalling pathways should be included in future analyses of the effects of MPS.

An own review article summarizes the current state of knowledge on the effects of MPS and synthetic glucocorticoids as used in about 10% of pregnant women to induce lung maturation in babies at risk of premature birth. Synthetic glucocorticoids have been shown to have a formative effect on the fetal vascular system, but no data on the long-term effects of MPS on the vascular system exist yet.

Nevertheless, parallels can be drawn between our effects of MPS and the effects of synthetic glucocorticoids and both may have negative effects on cardiovascular health in later life.

We were able to show multiple acute and characterizing effects of MPS on the functional and structural maturation of vascular systems involved in blood pressure regulation. The extent to which these effects contribute to a predisposition to cardiovascular disease must be demonstrated by studies of vasoreagibility in adult offspring. This work thus forms the basis for future innovative measures and recommendations for the prevention of the world's leading cause of death: cardiovascular diseases.

2 Zusammenfassung

Mütterlicher psychischer Stress (psychosozialer Stress (MPS) bei Tieren) während der Schwangerschaft, scheint ein wesentlicher Faktor für die Prädisposition von kardiovaskulären Erkrankungen im späteren Leben zu sein. Epidemiologische Daten zeigen, dass der Blutdruck von Kindern während der Schwangerschaft gestresster Mütter, erhöht ist. Zu den zugrundeliegenden Mechanismen ist bisher wenig bekannt.

Dies macht die Untersuchung der Mechanismen der stressbedingten Programmierung des kardiovaskulären Systems bei zunehmendem Stress in der Gesellschaft zu einer wichtigen Aufgabe unserer Zeit. In dieser Arbeit wurde am wichtigsten Tiermodell der menschlichen Fetalperiode, dem fetalen Schaf, erstmals die funktionelle und strukturelle Entwicklung wichtiger, an der Blutdruckregulation beteiligter, fetaler Gefäße und die Modulation der Reifung durch chronischen MPS in Abhängigkeit vom Schwangerschaftszeitpunkt untersucht. Die funktionelle Reifung von Vasodilatation und -konstriktion vermittelnden Gefäßmediatoren wurde mittels *small vessel wire myography* an distalen renalen Interlobulararteriolen und mesenterialen Widerstandsgefäßen untersucht. Die strukturelle Reifung der Blutgefäße wurde mittels Histologie bestimmt und die Expression an der Vasokonstriktion und -dilatation beteiligter Enzyme und Rezeptoren, immunhistochemisch untersucht.

Wir konnten ermitteln, dass die strukturelle Reifung beider Gefäßtypen zu Beginn des 3. Trimenons bereits abgeschlossen ist, mesenteriale Blutgefäße im letzten Trimenon jedoch noch funktionell reifen. Diese Reifung ist charakterisiert durch einen Anstieg der Vasoreagibilität aufgrund einer erhöhten Kontraktilität der Gefäße gegenüber K^+, Endothelin-1 und Noradrenalin und einer verstärkten Vasodilatation gegenüber Acetylcholin und Prostaglandin-E2. Insgesamt wurde die funktionelle Reifung nicht begleitet von einem Anstieg der Expression vasoreaktiver Rezeptoren (AChRM2, AChRM3, EP2, ET_A, ET_B, α_{1A}, β_2) bzw. Enzyme (eNOS, COX-2).

Der Einfluss von MPS auf die Gefäßreifung ist abhängig vom Zeitpunkt der Stressexposition. Unabhängig vom Zeitpunkt der Stressung führte MPS zu einer verzögerten Reifung der Gefäßmuskelzellen in mesenterialen aber nicht renalen Gefäßen, was anhand einer veränderten Myosinzusammensetzung deutlich wurde, sich jedoch nicht an einer Änderung des Gefäßdurchmessers (*media-to-lumen-ratio*) widerspiegelte.

Funktionell führte MPS im 1.-2. Trimenon zu einer Zunahme der Vasokonstriktion (Endothlin-1) in den mesenterialen Gefäßen und einer verstärkten Vasodilatation (Acetylcholin) in den renalen Gefäßen am Ende des 2. Trimenons. Diese Effekte wurden durch eine verminderte mesenteriale noradrenerg vermittelte Vasokonstriktion und eine verstärkte renale Vasodilatation (NO, Prostaglandin-E2) 30 Tage nach MPS, d.h. am Ende des 3. Trimenons ersetzt, möglicherweise um die initial stressbedingt erhöhte Vasokonstriktion zu kompensieren. MPS im 3. Trimenon beeinflusste die Vasokonstriktion nicht, sondern erhöhte die Vasodilatation (Acetylcholin) in den mesenterialen Gefäßen und verringerte die NO-vermittelte Antwort renaler Gefäße. Die funktionellen Stresseffekte waren unabhängig vom Gestationsalter nicht von einer Veränderung der untersuchten Rezeptor- bzw. Enzymexpression begleitet, was eine Modifikation intrazellulärer Signalwege durch MPS nahelegt. Daher sollte in zukünftige Analysen der Effekte von MPS die Untersuchung intrazellulärer Signalwege eingebunden werden.

Ein eigener Übersichtsartikel, fasst den aktuellen Wissensstand über die Effekte von MPS und synthetischen Glukokortikoiden zusammen, wie sie bei etwa 10% der Schwangeren zur Lungenreifeinduktion bei von Frühgeburt bedrohten Babies verwendet werden. Es zeigte sich, dass synthetische Glukokortikoide prägende Effekte auf das fetale vaskuläre System besitzen, jedoch noch keine Daten zu den langfristigen Auswirkungen von MPS auf das vaskuläre System existieren.

Trotzdem können Parallelen zwischen unseren Effekten von MPS und den Effekten synthetischer Glukokortikoide gezogen werden und beide können negative Einflüsse auf die kardiovaskuläre Gesundheit im späteren Leben haben.

Wir konnten damit multiple akute und prägende Effekte von MPS auf die funktionelle und strukturelle Reifung von in die Blutdruckregulation eingebundener Gefäßsystemen zeigen. Inwieweit diese Effekte zu einer Prädisposition für kardiovaskuläre Erkrankungen beitragen, müssen Untersuchungen der Vasoreagibilität an adulten Nachkommen zeigen. Diese Arbeit bildet somit die Grundlage, um in Zukunft innovative Maßnahmen und Empfehlungen für die Prävention der weltweit häufigsten Todesursache: den kardiovaskulären Erkrankungen ableiten zu können.

3 Einleitung

3.1 Kardiovaskuläre Erkrankungen – gibt es eine pränatale Disposition?

Kardiovaskulären Erkrankungen sind die häufigste Todesursache in den Industrienationen. Jährlich sterben rund 17.9 Millionen Menschen (Heart disease and stroke statistics 2018) an Herz-Kreislauf- und zerebrovaskulären Erkrankungen weltweit. Umweltfaktoren welche direkt auf das Individuum einwirken spielen bei der Krankheitsprädisposition eine wesentliche Rolle. Etablierte kardiovaskuläre Risikofaktoren sind ungesunde Ernährung, Nikotinabusus, Adipositas und exzessiver Alkoholkonsum (Ambrose et al. 2004, Reynolds et al. 2003, Van Gaal et al. 2006). Während der direkte Einfluss von negativen Umweltfaktoren auf das kardiovaskuläre Risiko unbestritten ist, vermehren sich die Hinweise, dass auch negative Umwelt- und Lebensstileinflüsse in der Schwangerschaft das kardiovaskuläre Risiko im Erwachsenenalter modifizieren können (McMillen & Robinson 2005).

Erste Hinweise auf eine Verbindung zwischen einer ungünstigen pränatalen Umgebung und einem erhöhten Risiko von kardiovaskulären Erkrankungen im späteren Leben fanden sich in Untersuchungen des Zusammenhanges zwischen Geburtsgewicht und kardiovaskulären Todesfällen in Großbritannien (Barker et al. 1989). Diese britische Kohorte umfasste 5654 Geburten der Geburtsjahrgänge 1911-30. Barker et al. beobachteten in dieser Kohorte eine erhöhte standardisierte Sterblichkeitsrate bei Männern mit einem geringen Geburtsgewicht (Barker et al. 1989). Dabei stiegt das Risiko eines frühzeitigen kardiovaskulär-bedingter Todesfalls (<65 Jahre) mit einem Geburtsgewicht unter 2495 g (Barker et al. 1993). Ein wesentlicher Beitrag eines erhöhten Risikos für Bluthochdruck und metabolischen Veränderungen wie eine Insulinresistenz im Erwachsenenalter, und damit der kardiovaskulären Mortalität, scheint ein unterdurchschnittliches Geburtsgewicht zu sein (Barker et al. 1997). Das niedrige Geburtsgewicht ist dabei nur ein Epiphänomen von umweltbedingten Veränderungen der fetalen Entwicklung (siehe Kap 3.2 Die Rolle von maternalem psychischem Stress und einer pränatalen Therapie mit synthetischen Glukokortikoiden). Diese Untersuchungen legten den Grundstein für ein besseres Verständnis der intrauterinen Prägung der postnatalen phänotypischen Entwicklung und

der Prädisposition für kardiovaskuläre und metabolische Erkrankungen (Schleußner et al. 2011, Bateson. et al. 2004). Aus diesen epidemiologischen und nachfolgenden experimentellen Untersuchungen ging eine wegweisende Hypothese hervor, welche zwischen einer ungünstigen intrauterinen Umgebung und einer Prädisposition für Erkrankungen im späteren Lebenzeinen Zusammenhang sieht ('Developmental Origins of Health and Disease (DOHaD) hypothesis') (Arima et al. 2018). Als sensitive Systeme gegenüber intrauterinen Umwelteinflüssen sind hierbei physiologische Systeme mit hoher Plastizität wie das fetale kardiovaskuläre System zu nennen, die aufgrund ihrer hohen und langanhaltenden Plastizität während der Schwangerschaft durch pränatale Umwelteinflüsse in Funktion und Struktur modifiziert bzw. geprägt werden können (McMillen and Robinson, 2005). Der Prozess der fetalen Prägung, wird als "fetal programming" bezeichnet und ist ein zentraler Punkt der Forschung in den letzten 30 Jahren.

Der biologische Sinn funktioneller Adaptionen im Sinne einer Fetalen Programmierung besteht offensichtlich darin, den Fetus auf ein Leben unter den zu erwartenden veränderten Umweltbedingungen, zum Beispiel auf ein vermindertes Nahrungsangebot vorzubereiten. Metabolischen Veränderungen, die zu einer Insulinresistenz im späteren Leben führen können, dienen zu einer besseren Nahrungsverwertung. Hierin liegt das Dilemma: Der Preis für die Adaptionen, die das Überleben sichern sollen ist langfristig eine Prädisposition genau dieser Organsysteme für Erkrankungen wie des kardiovaskulären Systems. Da der Preis in Form von Krankheiten erst nach der Reproduktionsphase der Nachkommen eingefordert wird, ist er für die Auslese nicht wirksam und wird aus biologischer Sicht gerne gezahlt.

Aufbauend auf den epidemiologischen Untersuchungen von Barker et al. 1993 wurden anfänglich die Auswirkungen einer intrauterinen Mangelernährung (z. B. durch mütterlichen Mangelernährung oder Plazentainsuffizienz) auf das kardiovaskuläre System des Fetus untersucht, welche in Form eines geringen Geburtsgewichtes in Erscheinung tritt (Barker et al. 1993). Hierbei zeigte sich, dass Mangelernährung mit einer veränderten Nephrogenese, der Entwicklung einer Insulinresistenz und einer Herzhypertrophie in Zusammenhang gebracht werden kann (McMillen & Robinson 2005).

Neben einer nutritiven Mangelversorgung durch mütterliche Mangelernährung oder plazentarer Dysfunktion, welche in einem niedrigeren Geburtsgewicht münden kann, scheinen andere Umwelteinflüsse eine wesentliche Rolle bei der Programmierung des fetalen Phänotyps kardiovaskulärer Erkrankungen im späteren Leben zu spielen. Diese umfassen eine erhöhte Exposition des Fetus gegenüber Stresshormonen, sei es durch mütterlichen Stress oder die therapeutische Applikation von synthetischen Glukokortikoiden (Plagemann et al. 2005). Diese Effekte und die zugrundeliegenden Mechanismen sind jedoch wesentlich geringer untersucht als die Effekte einer fetalen Mangelernährung. Die Optimierung stressspezifische Reaktionen wie Angst und Aufmerksamkeitsfokussierung in einer stressreichen Umgebung waren während der Evolution neben der optimalen Nährstoffausnutzung wichtige Adaptationsmechanismen für den Fetus, die das Überleben sicherten.

3.2 Die Rolle von maternalem psychischem Stress und einer pränatalen Therapie mit synthetischen Glukokortikoiden

10-15% aller schwangeren Frauen der Industrienationen und 10-41% in Ländern mit niedrigem und mittlerem Einkommen leiden an perinatalen psychischen Störungen (UNFPA 2008; Bennett et al. 2016). Neben psychischen Erkrankungen wie Depressionen und Angststörungen, welche mit einer erhöhten Hypothalamus-Hypophysen-Nebennieren (HPA)-Achsenaktivität und endogenem Hyperkortisolismus einhergehen, nimmt auch die Rate von maternalem psychischen Stress deutlich zu (UNFPA 2008; Falah-Hassani et al. 2017). Neben einem erhöhten Risiko von Frühgeburten und frühgeburtlicher Mortalität scheint mütterlicher psychischer Stress während der Schwangerschaft langfristige Auswirkungen auf die kardiovaskuläre Gesundheit der Nachkommen zu haben (van Dijk et al. 2012; Plana-Ripoll et al. 2016).

Maternaler psychischer Stress aktiviert die mütterliche HPA-Achse, eine autoregulierte, endokrine Kaskade. Die Aktivierung beginnt mit der Sekretion des Corticotropin-Releasing-Hormons (CRH) und führt über die Freisetzung von Adreno-Corticotropin aus der vorderen Hypophyse zu einer Ausschüttung von Cortisol aus der Nebennierenrinde, in dessen Folge der mütterliche Cortisolspiegel ansteigt (Ulrich-Lai & Herman et al. 2009). Die negative Rückkopplung der HPA-Achse durch den Cortisolspiegel reguliert das Maß und die Dauer der CRH-Freisetzung aus dem Hypothalamus (Smith et al. 2006).

Der Transfer von Cortisol zum fetalen Kreislauf erfolgt über die Plazenta, wobei die plazentare 11β-Hydroxysteroid-Dehydrogenase 2 (11β-HSD2) ca. 80-90 % des maternalen Cortisols bei der Passage inaktiviert (Abbildung 1). Das in den fetalen Kreislauf gelangende Cortisol stellt für den Fetus einen wesentlichen Stressor über den gesamten Zeitraum der Schwangerschaft dar, da Glukokortikoidrezeptoren schon früh während der Schwangerschaft exprimiert werden (Rose et al. 1985), obwohl der Fetus erst am Ende der Schwangerschaft selbst in der Lage ist, Cortisol zu produzieren (Fowden & Forhead et al. 2015).

Einen wesentlichen Beitrag zum Verständnis der programmierenden Mechanismen lieferten Untersuchungen zum Einfluss von synthetischen Glukokortikoiden auf die fetale Reifung. Synthetische Glukokortikoide sind kein Substrat für 11ß HSD 2 in der Plazenta und gehen deshalb ungehindert auf den Fetus über (Seckl & Meaney, 2004). Sie werden in der Geburtsmedizin regelhaft bei drohender Frühgeburt zur Prävention des Atemnotsyndroms eingesetzt (Schleußner et al. 2011). Der unbestrittene Benefit in Form verminderter frühgeburtliche Morbidität und Mortalität aufgrund einer Stimulierung der Reifung der Pneumozyten (Grier & Halliday et al. 2004) geht einher mit einem geringen Geburtsgewicht (Kutzler et al. 2004; Seckl, 2001). Dieser wachstumsverzögernde Effekt lässt sich nicht nur beim Menschen, sondern auch im Tiermodell, z.B. beim Schaf nachweisen (Reinisch et al. 1978; Kutzler et al. 2004). Eine Wachstumsstörung und das damit verbundene reduzierte Geburtsgewicht kann auch die Folge von MPS sein (Wadhwa et al. 1993).

Der wachstumsverzögernde Effekt endogener und synthetischer Glukokortikoide ist Ausdruck einer Inhibierung der Zellproliferation der mit der beschleunigten Zelldifferenzierung fetaler Gewebe einhergeht (Schleußner et al. 2011). Neben der Adaption physiologischer Systeme aufgrund einer veränderten strukturellen Reifung bewirken endogene und synthetische Glukokortikoide auch eine Veränderung der Reifung und damit der späteren Funktion physiologischer Systeme. Das Insulinsystem und die HPA-Achse sind hierfür typische Beispiele (siehe Kapitel 3.3.2 Glukokortikoidwirkung auf das fetale kardiovaskuläre System). Die hierdurch hervorgerufenen akuten und langfristigen kardiovaskulären Effekte sollen ebenfalls im folgenden Kapitel besprochen werden.

Glukokortikoide bewirken somit umfassende funktionelle und strukturelle Veränderungen fetaler Organe und die Aktivierung biochemischer Prozesse, um den Organismus auf die postnatale Umgebung vorzubereiten (Fowden et al. 1998). Diese vielfältigen Effekte lassen sich mit der nahezu ubiquitären Expression von Glukokortikoidrezeptoren im Organismus erklären.

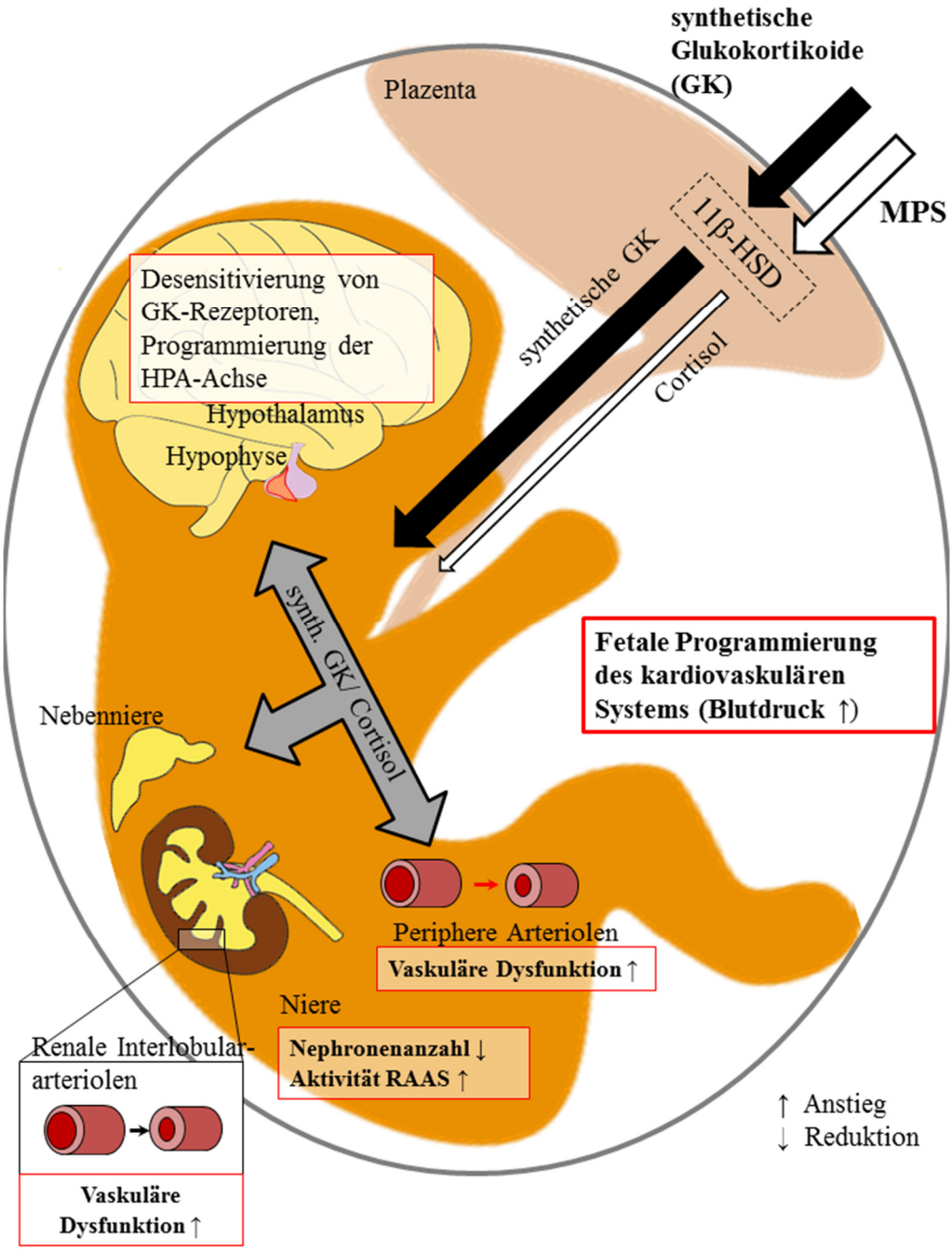


Abbildung 1. Der Einfluss von maternalem psychosozialen Stress (MPS) und einer pränatalen Exposition zu synthetischen Glukokortikoiden (GK) auf die fetale vaskuläre Entwicklung. Mütterliches Cortisol wird durch die plazentare 11βHSD2 zu 80-90% zum inaktiven Cortison konvertiert. Synthetische Glukokortikoide werden hingegen nicht inaktiviert und können den Fetus so in höherer Konzentration erreichen. Cortisol und synthetische Glukokortikoide haben einen Einfluss auf die Aktivität der Hyothalamus-Hyposphysen-Nebennieren (HPA)-Achse sowie die Niere und regulieren durch deren neuroendokrine/homeostatische Funktion den Blutdruck im fetalen Kreislauf. Die Exposition des Fetus gegenüber zu hohen mütterlichen Cortisolspiegeln/synthetischen Glukokortikoiden kann letztlich in eine vaskuläre Dysfunktion, eine verminderte Nephronenanzahl und eine Hyperaktivität der HPA-Achse des Fetus resultieren.

3.3 Programmierung des fetalen kardiovaskulären Systems durch MPS und einer Exposition zu synthetischen Glukokortikoiden

Aufgrund der nahezu ubiquitären Expression von Glukokortikoidrezeptoren im Organismus bewirken erhöhte fetale Glukokortikoidspiegel nicht nur umfassende funktionelle Veränderung in der fetalen Lunge, sondern auch in anderen fetalen Organsystemen, die zum Zeitpunkt der Glukokortikoidexposition reifen und eine hohe Plastizität besitzen, so auch im kardiovaskulären System (Fowden et al. 1998). Um diese besser zu verstehen, soll zunächst die physiologische Blutdruckregulation erläutert werden.

3.3.1 Physiologische Blutdruckregulation

Der arterielle Blutdruck wird durch kurz- bzw. mittelfristige Rückkopplungssysteme reguliert, welche auf autonome Nerven und zirkulierende Hormone als ihre Bestandteile angewiesen sind. An der Blutdruckregulation sind drei Mechanismen wesentlich beteiligt: kurzfristig durch neurale und hormonelle Reflexmechanismen, mittelfristig durch die HPA-Achse und das Renin-Angiotensin-Aldosteron-System (RAAS) und langfristig durch die Modulation zentraler und peripherer Komponenten des kardiovaskulären Systems (z.B. Veränderung der Salzaufnahme) (Dampney et al. 2002). Kurz- und mittelfristige Regulationsmechanismen beeinflussen den Blutdruck über Veränderungen der Herzleistung und den Strömungswiderstand des Blutes durch Vasokonstriktion (Engstellung) bzw. Vasodilatation (Weitstellung) von Widerstandsgefäßen. Eine wesentliche Komponente des peripheren vaskulären Widerstandes sind die mesenterialen Widerstandsgefäße. Sie realisieren die kurzfristige Blutdruckanpassung, initiiert durch das sympathoadrenerge Nervensystem (Cowley et al. 1992). Vermittelt durch periphere Rezeptoren (z.B. Barorezeptoren, Chemorezeptoren) ist das sympathische Nervensystem kurzfristig in der Lage den arteriellen Blutdruck anzupassen, indem der Strömungswiderstand des Blutes in den peripheren Widerstandsgefäßen durch sympathetische Nervenfasern reguliert wird (Dampney et al. 2002). An der mittelfristigen Blutdruckregulation sind die distalen renalen Interlobularteriolen beteiligt, welche über die Kontrolle des kortikalen renalen Blutfluss die glomeruläre Filtration kontrollieren (Hanson, 1993; Robillard & Nakamura,

1988). Das RAAS reguliert, aktiviert durch hämodynamische Reize, den kortikalen renalen Blutfluss und mittels der Modulation der Natrium- und Wasserretention auch das Blutvolumen (Robillard & Nakamura, 1988).

In beiden in dieser Arbeit untersuchten Gefäßsystemen, den mesenterialen Widerstandsgefäßen und renalen distalen Interobulararteriolen, wirken spezifische Mediatoren vasokonstriktiv (verengend, allg. blutdrucksteigernd), andere vasodilatativ (weitend, allg. blutdruckmindernd) auf die glatten vaskulären Muskelzellen (Abbildung 2).

Am Gefäßmuskel potenzieren Glukokortikoide die Vaskonstriktion über die vermehrte Expression verschiedener Rezeptoren und Second-Messenger-Systeme von Vasokonstriktoren wie Noradrenalin, Angiotensin II und Endothelin-1 und eine Erhöhung der intrazellulären Ca^{2+}-Konzentration (Abbildung 2). Über Second Messenger wird die Aktivität der Myosin-Leichte-Kette-Kinase erhöht, was eine Konstriktion der glatten Muskulatur zur Folge hat (Abbildung 2). Möglich ist ebenso eine Beeinflussung der N^{+}-K^{+}-ATPase (Ullian et al. 1999), welche durch die Erhöhung der extrazellulären K^{+} -Konzentration zur Depolarisation der Zellmembran und damit zur Vasokonstriktion führt. Desweitern inhibieren Glukokortikoide im Gefäßmuskel die Expression der an der Vasodilatation beteiligten Ionenkanäle (Brem et al. 1999). Langfristig tragen erhöhte Glukokortikoidspiegel über eine Hypertrophie der Gefäßmuskelzellen zur Fixierung des erhöhten Vasotonus bei (Ullian et al. 1999). Am Endothel vermitteln Glukokortikoide eine verminderte endothelabhängige Vasodilatation über die Beeinflussung der NO- und Prostaglandin E2- (PGE2) Synthese (Ullian et al. 1999) (Abbildung 2).

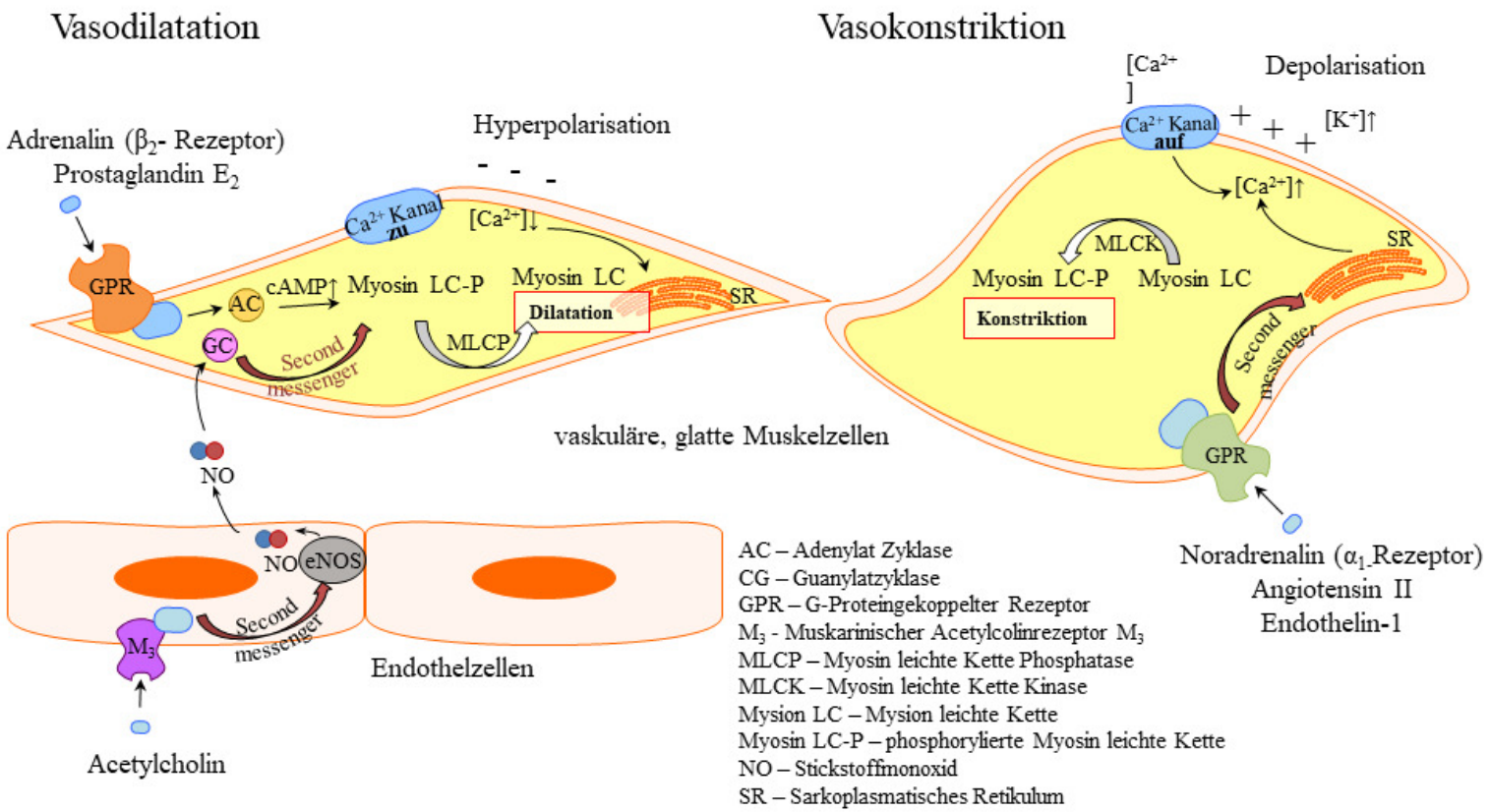


Abbildung 2. Zelluläre Prozesse in vaskulären glatten Muskelzellen und Endothelzellen während der Vasokonstriktion und Vasodilatation. NO vermittelt eine endothelunabhänige Vasodilatation durch die Bindung an die lösliche Guanylat-Zyklase. Acetylcholin bindet an den muskarinischen Acetylcholinrezeptor M3 der Endothelzellen und vermittelt über NO die Dilatation der glatten Muskelzelle. Prostaglandin-E2 bindet an den G-protein-gekoppelten Membranrezeptor, was die Adenylatzyklase aktiviert und über second messenger eine Dilatation der glatten Muskelzelle hervorruft. Noradrenalin, Endothelin-1 und Angiotensin vermitteln über die Bindung an spezifische G-protein-gekoppelter Membranrezeptoren den Anstieg der intrazellulären Ca^{2+} Konzentration, was zur Konstriktion der glatten Muskelzelle führt.

3.3.2 Glukokortikoidwirkung auf das fetale kardiovaskuläre System

Der Einfluss von synthetischen Glukokortikoiden wurde eingehend am Tiermodell des fetalen Schafes untersucht. Akut reduzieren synthetische Glukokortikoide im Tiermodell des fetalen Schafes (Kapitel 3.4) die Durchblutung des fetalen Gehirns (Schwab et al. 2000) und steigern über Angiotensin II-vermittelte Mechanismen den peripheren Gefäßwiderstand, was zu einer Erhöhung des systemischen fetalen Blutdrucks führt (Tangalakis et al. 1992; Quaedackers et al. 2005, Docherty et al. 2001; Derks et al. 1997).

Ein programmierender Effekt auf die Blutdruckregulation nach pränataler synthetischer Glukokortikoidexposition im Sinne einer Blutdrucksteigerung ist im Schafmodell im juvenilen bzw. adulten Nachkommen nachweisbar (Dodic et al. 1998, Peers et al. 2001, Roghair et al. 2005, Wintour et al. 2003). Auch in Nagetiermodellen ist ein erhöhter Blutdruck nach der pränatalen Glukokortikoidexposition im Erwachsenenalter beschrieben, was für eine spezies-übergreifenden Effekt der Programmierung der Blutdruckentwicklung spricht (Ratte: Celsi et al. 1998, Meerschweinchen: Banjanin et al. 2004, Schaf: Wintour et al. 2003, Dodic et al. 2001, Peers et al. 2001).

Bekannt ist, dass hohe fetale Cortisolspiegel eine prägende Wirkung auf die Niere, und blutdruckregulierende hormonelle Systeme wie das RAAS und die HPA Achse besitzen (Moritz et al. 2002) (Abbildung 1). Langfristig bewirken erhöhte Glukokortikoidspiegel während der fetalen Reifung der HPA-Achse eine persistierende Sollwertverstellung der Glukokortikoidrezeptorsensitivität im Hippocampus. Die Desensitivierung der in die negative Rückkopplung der HPA-Achse involvierten Glukokortikoidrezeptoren wird bedingt durch epigenetische Veränderungen am Glukokortikoidrezeptor auf der Basis von DNA-Methylierungsprozessen (Pariate et al. 2001, Weaver et al. 2004) und führt zu einem anhaltenden Hyperkortisolismus (Holboer et al. 2001). Die dadurch induzierte evolutionär gewollte Optimierung stressspezifischer Reaktionen geht mit einem erhöhten Risiko für stressassoziierte Erkrankungen wie den kardiovaskulären Erkrankungen einher. Chronisch erhöhte Blutcortisolspiegel bedingen außerdem arteriellen Bluthochdruck, und erhöhen so langfristig das kardiovaskuläre Risiko (Wintour & Moritz et al. 1997).

Die Entwicklung eines gestörten Glukosestoffwechsels aufgrund einer Insulinresistenz mit konsekutivem metabolischen Syndrom (Bertram & Hanson, 2001) ist ebenso als kardiovaskuläre Risikofaktor eine Folge der pränatalen Exposition zu Glukokortikoiden.

Neben der Niere sind periphere Widerstandsgefäße, welche wesentlich an der Blutdruckregulation beteiligt sind (siehe Kapitel 3.3.1 Physiologische Blutdruckregulation), vulnerabel gegenüber einer pränatalen Exposition zu synthetischen Glukokortikoiden. Der Effekt von synthetischen Glukokortikoiden auf die Prägung des fetalen kardiovaskulären Systems lässt sich durch die Entwicklung einer vaskulären Dysfunktion erklären (Wintour et al. 2003) (Abbildung 1). Die vaskuläre Dysfunktion gilt indes als Indiz für die Steigerung des vaskulären peripheren Widerstandes (verringerte Vasodilatation, verstärkte Vasokonstriktion), welcher erst im späteren Leben durch einen erhöhten Blutdruck oder einer Herzhypertrophie ersichtlich wird (Nuyt 2008).

Die Effekte auf Vasomediatoren waren zwischen den Studien heterogen. Reproduzierbar zeigte sich, dass durch die Applikation synthetischer Glukokortikoide die Vasokonstriktion in Response auf Endothlin-1 steigt (Docherty et al. 2001, Molnar et al. 2003, Molnar et al. 2002) und die endothelvermittelte Vasodilatation in Reaktion auf Acetylcholin (Anwar 1999, Roghair 2004, Molnar 2003) verringert wird.

Pränatal erhöhte Glukokortikoidspiegel prägen damit die Blutdruckregulation auf der endokrinen, renalen und vaskulären Ebene und tragen somit multifaktoriell zur Programmierung der Blutdruckdysregulation im Erwachsenenalter bei (Abbildung 1). In wie weit sich endogen erhöhte Glukokortikoidspiegel, als Folge von MPS, auf die Prägung fetaler Systeme auswirken, ist jedoch nicht hinreichend untersucht. Diese Arbeit widmet sich nun der Frage ob die funktionellen und /oder strukturellen Programmierung es vaskulären Systems durch MPS, mit den Glukokortikoid-vermittelten Effekten vergleichbar sind.

3.4 Das fetale Schaf als Modell der Fetalphysiologie

Da das subjektive Erleben von Stress schwer zu quantifizieren ist und sich aus ethischen Gründen eine Exposition der werdenden Mütter gegenüber experimentellen Stressoren verbietet, ist die Untersuchung der Mechanismen von psychischem Stress auf eine fetale Programmierung von kardiovaskulären Erkrankungen im späteren Leben nur im Tiermodell möglich. Das klassische Modell für die menschliche Fetalperiode ist das fetale Schaf. Im Gegensatz zu kleineren Versuchstieren wie Nagern, die extrem unreif zur Welt kommen (Andersen et al. 2018), ähnelt der zeitliche Ablauf der intrauterinen Entwicklung des Schaffeten der des menschlichen Feten. Die Entwicklung wesentlicher in der

Blutdruckregulation involvierter Organsysteme findet in beiden Spezies *in utero* statt. So ist z.B. die humane Nephrogenese zwischen der 34-35 Schwangerschaftswoche, im Schaf ca. am 130. Schwangerschaftstag abgeschlossen (Wintour and Moritz, 1997; Hinchliffe et al. 1992; Mackenzie & Brenner, 1995). Auch die Autoregulation der Durchblutung von Gehirn, Niere und Darm des Schafes ist bereits vor der Geburt ausgeprägt, was Parellelen zur Reifung des humanen Gefäßsystems verdeutlicht (Buckley 1986).

An diesem Tiermodell wurde unter anderem die pränatale Glukokortikoid-Therapie zur Förderung der Lungenreifung bei von Frühgeburt bedrohten Kindern entwickelt (Liggins et al. 1972) und wesentliche grundlegende Erkenntnisse der fetalen Programmierung von Erkrankungen im späteren Leben gewonnen (Anwar et al. 1999, Ruppecht et al. 2009, Tangalakis et al. 1992). Es wurden in diesem Tiermodell auch umfangreiche Erkenntnisse zu den akuten und langfristigen Effekten von Glukokortikoiden und MPS, dem Äquivalent zu maternatelm psychischem Stress beim Menschen, (Liggins et al. 1972; Rakers et al. 2013) auf die fetale Reifung gewonnen.

In der Arbeitsgruppe „Fetale Programmierung von Erkrankungen im späteren Leben“ unter Leitung von Prof. Dr. Matthias Schwab wurden in diesem Tiermodell experimentelle Paradigmen entwickelt, die Effekte von MPS und synthetischen Glukokortikoiden auf die Entwicklung des Fetus und die Programmierung von Krankheiten im späteren Leben zu untersuchen und die Mechanismen der Stressübertragung von der Mutter auf den Fetus zu charakterisieren. Als artspezifischer Stressor fungiert die wiederholte Isolation des trächtigen Muttertieres von der Herde, welche nachweislich mit einem wiederkehrendem maternaler Cortisolanstieg (Rakers et al. 2013) einhergeht und so die Konstanz eines chronischen Stressors ohne wesentliche Habituation erfüllt (Abbildung 3). Unterschiedliche Zeitpunkte der Stressexposition während der Schwangerschaft ermöglichen zusätzliche die Identifikation besonders MPS-vulnerabler Phasen in der Schwangerschaft.

Hierbei scheint insbesondere die frühe Schwangerschaft eine MPS-vulnerable Episode darzustellen. So führt chronischer, MPS während des ersten Trimenons zu Adaptation des fetalen Organismus an Stress, was sich in der Hyperaktivität der fetalen HPA-Achse im dritten Trimenon wiederspiegelt (Dreiling et al. 2018; Rakers et al. 2013, Rakers et al. 2017). Akuter MPS bewirkt im Feten eine Steigerung systemischen Blutdrucks welcher mit sympathoadrenergen Aktivierung und vermehrter Cortisolausschüttung assoziiert ist

(Rakers et al. 2013). Diese fetale kardiovaskuläre Stressreaktion ist nach einer chronischen Stressexposition verstärkt. Die akute fetale Blutdruckreaktion während der Stressexposition belegt eine vaskuläre Adaptation des Fetus an den akuten Stressor. Die Charakterisierung der Mediatoren die dieser Blutdruckregulation auf vaskuläre Ebene zugrunde liegen, sowie die Detektion MPS- vulnerabler Phasen in der Schwangerschaft für anhaltender Veränderungen der fetalen Vasoreagibilität sollen in der aktuellen Studie untersucht werden.

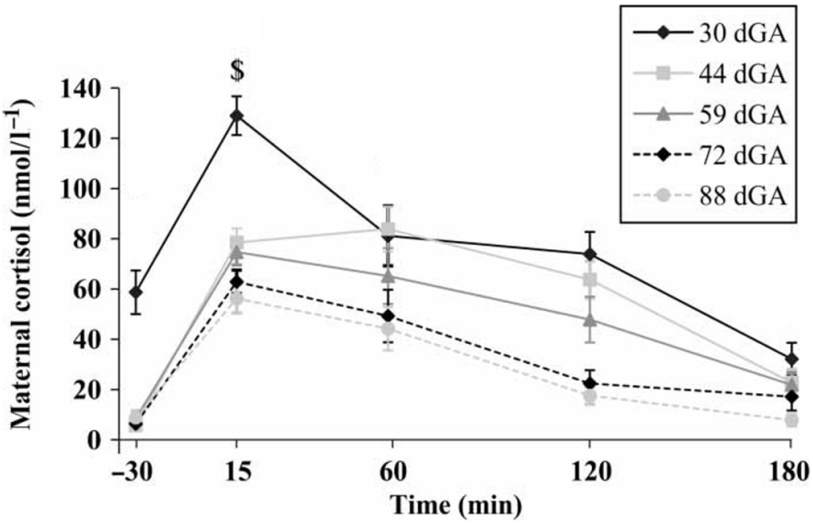


Abbildung 3: Maternale Cortisol-Spiegel im venösen Blut von schwangeren Schafen nach wiederholter Isolation des Muttertieres von der Herde für 3 Stunden, zweimal wöchentlich (30-130 days gestation (dGA) = Schwangerschaftstage, n=18). Werte sind Mittelwerte ± SEM. (Rakers et al. 2013)

3.5 Ziele der Arbeit

Übergeordnetes Ziel der Arbeit war **die Untersuchung von maternalem psychozialem Stress (MPS) auf die Reifung des vaskulären Systems.** Die spezifischen Ziele der Arbeit waren folgende:

1) Die Charakterisierung der funktionellen und strukturellen Entwicklung wichtiger an der Blutdruckregulation beteiligter Gefäßsysteme im Tiermodell des fetalen Schafes als Basis für nachfolgenden Untersuchungen zum MPS. Der Fokus lag dabei auf den zwei wesentlichen in die Blutdruckregulation eingebundenen Gefäßsystemen - den mesenterialen Arteriolen, welche als Vertreter der peripheren Widerstandsgefäße wesentlich an der kurzfristigen Blutdruckanpassung beteiligt sind und den distalen renalen Interlobulararteriolen, welche über die Kontrolle des kortikalen, renalen Blutflusses die Aktivität des RAAS und die glomeruläre Filtration modulieren und damit den Blutdruck kontrollieren.

Folgende Arbeitshypothesen wurden getestet:

- Die Reifung der mesenterialen Arteriolen und der renalen Interlobulararteriolen erfolgt zu unterschiedlichen Phasen der Schwangerschaft.
- Die funktionelle und strukturelle Reifung von renalen Interlobulararteriolen beginnt bereits im 2. Trimenon, parallel zur bekannten physiologischen Entwicklung der Nieren.
- Die funktionelle und strukturelle Reifung der mesenterialen Arteriolen verläuft im letzten Trimenon der Schwangerschaft, was sich in dem bekannten Anstieg des peripheren Widerstandes und systemischen Blutdruckes im letzten Drittel der Schwangerschaft wiederspiegelt.

2) Den Einfluss von MPS auf die funktionelle und strukturelle Reifung dieser fetalen Gefäßsysteme zu untersuchen. Hinweise auf funktionelle und strukturelle Veränderungen fetaler Gefäße lieferten Studien nach pränataler Exposition zu synthetischen Glukokortikoiden (Manuskript 3).

Folgende Arbeitshypothesen wurden getestet:

- Auf struktureller Ebene verändert MPS die Zusammensetzung des fetalen/adulten Myosins als möglicher Mechanismus für die Adaptation des Gefäßsystems an die pränatale Glukokortikoidexposition.
- Durch MPS werden spezifische Glukokortikoid-sensitive Vasotransmittersysteme beeinflusst (Endothelin-1, Acetylcholine, NO, Prostaglandine), im Speziellen die funktionelle Reifung des Endothels, die zu einem erhöhten Vasotonus im späteren Leben führen kann.

3) Die Identifizierung von vulnerablen Phasen von MPS auf die Entwicklung fetaler renaler und mesenterialer Blutgefäße.

Folgende Arbeitshypothese wurde getestet:

Aufgrund ihrer potenziell unterschiedlichen Reifungszeitpunkte während der Schwangerschaft (siehe Hypothese 1) hat MPS:

- im 1. und 2. Trimenon primär einen Einfluss auf die renale Gefäßentwicklung,
- im letzten Trimenon Auswirkungen auf die mesenteriale Gefäßentwicklung.

4) Die Erstellung eines Übersichtsartikels (Review), welcher den aktuellen Wissensstand über die prägenden die Effekte von MPS auf das kardiovaskuläre System zusammenfasst und den Effekten von pränataler Exposition zu synthetischen Glukokortikoiden gegenüberstellt.

Alle praktischen Untersuchungen wurden am etablierten Modell des fetalen Schafes durchgeführt. Es wurden die *akuten Auswirkungen* von chronischem MPS im 1. und 2. Trimenon (0.2-0.7 der Gestation) und im letzten Trimenon (0.7-0.9 der Gestation) untersucht, sowie der *prägende Effekt* von MPS im 1. Und 2. Trimenon, 30 Tage nach der Stressexposition. Die funktionellen Untersuchungen an mesenterialen Arteriolen und distalen renalen Interlobulararteriolen wurden unter Anwendung der *small vessel wire myography* durchgeführt. Strukturelle Analysen der Gefäße erfolgten mittels Histologie und Immunhistochemie.

4 Manuskripte

4.1 Manuscript 1: Fetal sheep mesenteric resistance arteries: functional and structural maturation

Research Paper

Journal of Vascular Research

J Vasc Res 2017;54:259–271
DOI: 10.1159/000477629

Received: June 8, 2016
Accepted after revision: May 14, 2017
Published online: August 16, 2017

Fetal Sheep Mesenteric Resistance Arteries: Functional and Structural Maturation

Julia J. Müller[a] Matthias Schwab[a] Charles R. Rosenfeld[c]
Iwa Antonow-Schlorke[a] Peter W. Nathanielsz[d] Florian Rakers[a]
Harald Schubert[b] Otto W. Witte[a] Sven Rupprecht[a]

[a]Hans Berger Department of Neurology and [b]Institute of Laboratory Animal Sciences and Welfare, Jena University Hospital, Jena, Germany; [c]Division of Neonatal-Perinatal Medicine, University of Texas Southwestern Medical Center, Dallas, TX, and [d]Wyoming Pregnancy and Life Course Health Center, University of Wyoming, Laramie, WY, USA

Keywords
Sheep · Fetus · Vascular development

Abstract
Background: Fetal blood pressure increases during late gestation; however, the underlying vascular mechanisms are unclear. Knowledge of the maturation of resistance arteries is important to identify the mechanisms and vulnerable periods for the development of vascular dysfunction in adulthood. ***Methods:*** We determined the functional and structural development of fetal sheep mesenteric resistance arteries using wire myography and immunohistochemistry. ***Results:*** Media mass and distribution of myosin heavy-chain isoforms showed no changes between 0.7 (100 ± 3 days) and 0.9 (130 ± 3 days) gestation. However, from 0.7 to 0.9 gestation, the resting wall tension increased accompanied by non-receptor-dependent (potassium) and receptor-dependent (noradrenaline; endothelin-1) increases in vasocontraction. Angiotensin II had no contractile effect at both ages. Endothelium-dependent relaxation to acetylcholine and prostaglandin E_2 was absent at 0.7 but present at 0.9 gestation. Augmented vascular responsiveness was paralleled by the maturation of sympathetic and sensory vascular innervation. Non-endothelium-dependent relaxation to nitric oxide showed no maturational changes. The expression of vasoregulator receptors/enzymes did not increase between 0.7 and 0.9 gestation. ***Conclusion:*** Vascular maturation during late ovine gestation involves an increase in resting wall tension and the vasoconstrictor and vasodilator capacity of the mesenteric resistance arteries. Absence of structural changes in the tunica media and the lack of an increase in vasoregulator receptor/enzyme expression suggest that vasoactive responses are due to the maturation of intracellular pathways at this gestational age.

Introduction

Maturation of the cardiovascular system during fetal life is a crucial component of adaptive development. It is characterized by an increase in peripheral vascular resistance and systemic blood pressure which mainly occur during the last third of the period of gestation, in preparation for postnatal life [1, 2]. Whereas an increase in vessel

KARGER

E-Mail karger@karger.com
www.karger.com/jvr

Dr. Sven Rupprecht
Hans Berger Department of Neurology
Jena University Hospital
Am Klinikum 1, DE–07747 Jena (Germany)
E-Mail Sven.Rupprecht@med.uni-jena.de

size, basal vasomotor tone, and neurohumoral vascular responsiveness during late gestation has been described for adrenal, cerebral, and renal arteries [3–5], the ontogeny of the function of the peripheral resistance arteries is poorly understood. Knowledge on the time course of maturational processes in resistance arteries is vital for identifying mechanisms and vulnerable periods for the development of vascular dysfunction. Such data are a prerequisite for shaping preventive strategies for the development of arterial hypertension and cardiovascular disease in later life.

Using the fetal sheep model, we analyzed the functional and structural maturation of mesenteric resistance arteries as major components of the vascular resistance system substantially involved in the regulation of blood pressure [6]. We performed our studies in fetal sheep at 0.7 (100 ± 3 days) and 0.9 (130 ± 3 days) gestation when the increase in peripheral vascular resistance and systemic blood pressure is greatest [1, 2]. The fetal sheep is a well-established animal model for studying the physiology of development since it is believed to most closely represent the human situation [7]. Using small-vessel wire myography, we systematically examined vascular responsiveness to the major vasoconstrictors and vasodilators that are crucial for the maintenance of basal vascular tone and adaptive vasoreactivity during fetal and postnatal life. Structural and regulatory pathways of vascular development were examined by evaluating the media mass, the expression of fetal and adult smooth-muscle myosin heavy-chain isoforms (as major contractile filaments), and the expression of receptors and enzymes involved in the control of vascular tone. We further analyzed the maturation of mesenteric sympathetic and sensory innervation as major regulators of mesenteric vascular tone and adaptive vascular control. For these analyses, we used immunohistochemistry since it allows for the localization of nerve fibers and receptor and enzyme expression in the compartments of small resistance arteries in the adventitia, media, and endothelium.

Methods

Animals

All procedures were approved by the Animal Welfare Committee of Thuringia. Twelve long-wool Merino ewes were bred on a single occasion and underwent Cesarean delivery at 0.7 gestation (100 ± 3 days gestation, term = 150 days, $n = 6$) and at 0.9 gestation (130 ± 3 days gestation, $n = 6$). All fetuses had a normal weight (0.7 gestation 950 ± 163 g; 0.9 gestation 3,612 ± 192 g). Anesthesia was induced by intramuscular injection of 1 g ketamine (Ketamin-Hydrochlorid®, Pfizer, Berlin, Germany) and 0.2 mg/kg midazolam (Midazolam-Hameln®, Hameln Pharmaceuticals, Hameln, Germany) and maintained with 4% isoflurane (Isofluran-Actavis®, Actavis, Munich, Germany). Fetuses were killed by rapid exsanguination while still under general anesthesia.

Mesenteric Artery Function

Artery Preparation

The small intestine, including the root of the mesentery, was removed and stored in ice-cold physiological saline solution (PSS, pH 7.4: NaCl 119 mM, KCl 4.7 mM, $CaCl_2$ 2.5 mM, $MgSO_4$ 1.17 mM, $NaHCO_3$ 25 mM, KH_2PO_4 1.18 mM, EDTA 0.03 mM, glucose 5.5 mM) for transport purposes for approximately 10 min. All chemicals were obtained from Sigma-Aldrich (Steinheim, Germany), unless stated otherwise. Third-branch mesenteric arteries were carefully dissected from the surrounding tissue under the stereo microscope (approx. 10-fold magnification) and transected into 2-mm-long segments. Arterial segments (internal diameter, approx. 400 µm) were threaded on 40-µm-diameter stainless-steel wires, taking care not to damage the vessel structure or endothelium. Segments were transferred into a 5-mL-volume chamber and connected to an isometric force transducer (Multi Wire Myograph, Model 610M, DMT, Aarhus, Denmark). Arteries were equilibrated in PSS, warmed to 37°C, and gas-flushed with 95% O_2 and 5% CO_2. Data were digitally recorded using the WINDAQ data acquisition system (WINDAQ, DATAQ Instruments, Akron, CA, USA).

Wire Myography

The normalization was performed by distending the arterial segment stepwise and measuring internal circumference (IC_i) and wall tension (T_i), respectively. IC_{100} (i.e., IC_i at the physiological transmural pressure of 13.3 kPa [100 mm Hg]) was calculated by plotting T_i against IC_i and calculating the point of intersection with the isobar curve at 100 mm Hg using the Laplace relation. The normalized internal diameter (L_{100}) was calculated by dividing the normalized IC_i by π [8]. In a pretest experimental setting using a representative sample of 5 artery segments at both fetal ages, the vascular response to depolarizing potassium solution (125 mM KPSS, equimolar substitution of NaCl with KCl in PSS) was obtained at different transmural pressure levels (0.5, 0.7, and 0.9 × L_{100}) to determine optimal vascular responsivness (not shown). Vascular response to KPSS was maximal when normalized IC_i was set to 0.9 × IC_{100}, which is comparable to previous studies on rat and fetal ovine resistance arteries [3, 8]. In agreement with the literature, L_{100} was set to 0.9 × IC_{100} during the normalization procedure at the start of each experiment.

Resting T_i was obtained after reaching a stable baseline level at least 30 min after normalization. Vascular and endothelial integrity were ascertained using a KPSS and 10^{-5} M noradrenaline (NA). Arteries showing an inadequate vasoconstrictor response to KPSS containing 10^{-5} M NA (i.e., not reaching the transmural pressure of 0.9 × 13.3 kPa (100 mm Hg) were not considered for the experiments [9]. Vasoconstrictor cumulative concentration-response curves were obtained for K^+ (1.25–120 mM), NA (10^{-10} – 5×10^{-4} M), endothelin-1 (ET-1; 10^{-10} – 5×10^{-7} M) and angiotensin II (ANGII; 10^{-12} – 5×10^{-6} M). Cumulative concentration-response curves for acetylcholine (Ach; 10^{-10} – 5×10^{-4} M), prostaglandin E_2 (PGE_2; 10^{-15} – 5×10^{-8} M) and the nitric oxide (NO)-donor sodium nitroprusside (SNP, 10^{-12} – 5×10^{-5} M) were obtained after precontraction with 5×10^{-6} M NA. Higher concentrations of the cor-

responding vasoactive agents were only added if the vascular tension showed a stable response plateau to the previous concentration. All vasoactive agents were dissolved in water.

Vasoconstrictor concentration-response curves to K^+ were normalized to IC_i and vessel length (N/m^2). Concentration-response curves to NA, ET-1, and ANGII were normalized to the maximal KPSS response ($\%K_{max}$). Vasodilator response curves were normalized to stable precontraction levels ($\%R_{max}$). The maximal vasoconstrictor response (C_{max} or $\%K_{max}$) or maximal relaxation ($\%R_{max}$) and the sensitivity (half the maximal effective concentration, EC_{50}) were calculated for all agents. EC_{50} is given as $-\log(EC_{50})$ for NA, ET-1, ACh, PGE_2, and SNP.

Data were analyzed by the same investigator blinded to group affiliation.

Mesenteric Artery Morphology

Histological Processing

We used immunohistochemistry since it allows for the localization of receptor and enzyme expression in the media and endothelium. Structural vascular development was analyzed by the media cross-sectional area (CSA) and the media-to-lumen ratio, reflecting changes in media mass and relative organization of the media around the lumen as well as the expression of fetal and adult smooth-muscle myosin heavy-chain isoforms (fetal: MHC-B; adult: SM2) [10]. Expression of ET-1 receptor types A and B (ET_A and ET_B), adrenoreceptors α_{1A}, α_{2A}, and β_2, muscarinic ACh receptors M2 and M3, (AChRM2 and AChRM3), PGE_2 receptors (EP_2 and EP_4), and vasoactive enzymes (endothelial NO synthase [eNOS] and cyclooxygenase-2 [COX-2]) were determined in the vascular smooth-muscle cells (VSMCs) located in the media and endothelium. The sensory and sympathetic innervation of the mesenteric arteries was determined using antibodies against calcitonin gene-related peptide (CGRP) for the sensory nerve fibers and against tyrosine hydroxylase (TH) for the sympathetic nerve fibers.

Multiple samples were taken from at least 6 fetal third-branch ovine mesenteric resistance arteries (400-µm internal diameter) at both gestation time points. Arterial segments were fixed in neutral-buffered 4% paraformaldehyde for at least 1 week and embedded in paraffin. Adjacent 6-µm sections were stained using polyclonal antibodies against AChRM2 (rabbit anti-AChRM2, 1:1,000; SP4407P, Acris Antibodies, Herford, Germany), AChRM3 (rabbit-anti-AChRM3, 1:100, ab60981, Abcam, Cambridge, UK), eNOS (mouse anti-eNOS, 1:500, 610296, BD Laboratories, Heidelberg, Germany), adrenoreceptor α_{1A} (rabbit anti-adrenoreceptor α_{1A}, 1:200, SP 5126P, Acris Antibodies), adrenoreceptor α_{2A} (rabbit anti-adrenoreceptor α_{2A}, 1:150, TA313317, Acris Antibodies) adrenoreceptor β_2 (rabbit anti-adrenoreceptor β_2, 1:100, ABIN498188, Antibodies-online, Aachen, Germany), CGRP (mouse anti-CGRP, 1:100, TA502495, Acris Antibodies), COX-2 (rabbit anti-COX-2, 1:600, LS-B5206, LifeSpan Bioscience, Eching, Germany,), ET_A (rabbit-anti ET_A, 1:250, G094–1, Assay Biotech, Sunnyvale, CA, USA), ET_B (rabbit-anti-ET_B, 1:700, SP4124P, Acris), EP_2 (rabbit anti-EP_2, 1:100, bs-4196R, Bioss, Woburn, MA, USA), EP_4 (rabbit anti-EP_4, 1:10, ab133170, Abcam), MHC-B (rabbit-anti-MHC-B, 1:250 [10]), SM2 (rabbit-anti-SM2, 1:800 [10]), and TH (rabbit anti-TH, 1:100, ab137721, Abcam) using the avidin-biotin-peroxidase complex method (Vectastain® elite ABC kit, Vector Laboratories, Peterborough, UK). After dewaxing, heat-induced antigen retrieval was performed using sodium citrate buffer for staining AChRM2, α_{1A}-adrenoreceptor, eNOS, and COX-2 (15 min in a microwave at 750 W) and for detecting AChRM3, adrenoreceptor α_{2A}, adrenoreceptor β_2, EP_2, EP_4, CGRP, and TH (pH 6.0, 20 min at 80°C). Sections were first treated with 0.4% H_2O_2 for 20 min to block tissue peroxidase activity, followed by 1 mM $NaBH_4$, and then incubated in 1.5% normal horse or goat serum (Vectastain), respectively. The primary antibody was added overnight (4°C), followed by a secondary biotinylated antibody for 24 h (1:200, 4°C; Vectastain). Sections were incubated in a preformed avidin-horseradish-peroxidase complex (Vectastain) for 1 h at 37°C. Immunostaining was made visible by precipitation of diaminobenzidine (3.3′-diaminobenzidine tablets) as a substrate for peroxidase (20 min at 21°C). For negative controls, the primary antibodies were substituted by normal goat or horse serum. The concentration of each antibody was optimized by serial dilutions to achieve the optimal staining results. Finally, 8 adjacent sections were stained with hematoxylin-eosin (HE) for morphometric analysis and to test the integrity of the vascular layers of mesenteric resistance arteries. When several arteries were present on the section, they were included in the analysis and the values were averaged.

Image Analysis of Immunostaining

The positive immunoreactivity (IR) for AChRM2, AChRM3, eNOS, COX-2, the α_{1A}, α_{2A}, and β_2 adrenoreceptors, ET_A, ET_B, EP_2, and EP_4 was used to assess the respective protein expression in the media and endothelium on a semiquantitative level. Light-microscopic images of the stained sections were digitized (×125 or ×250) using a 3-charge-coupled device color video camera (Axiocam HR, Zeiss, Jena, Germany) under standardized conditions. In each section, the whole area of the media and endothelium and the positive-immunostained area of these cell layers were assessed for each specific antibody, using an image analysis program (Scion Image 6.21, NIH, USA). The percentage of area positive for IR was calculated for each layer. When several arteries were present on the section, they were included in the analysis and values were averaged. Artery wall and lumen areas were measured in HE-stained sections, and the media CSA and media-to-lumen ratio were calculated to determine arterial media mass and relative organization of the media around the lumen [11, 12]. To address potential changes between medial (M) and endothelial (E) protein expression, the ratio between the percentages of area positive for IR of both layers was calculated (E/M ratio).

Evaluation of the histological sections was performed by the same investigator blinded to group affiliation.

Sympathetic and sensory innervation between mesenteric arteries at 0.7 and 0.9 gestation was assessed from the light-microscopic images of the TH and CGRP-stained sections, respectively. Photographs were taken with the Axiocam HR (×125 or ×250). Nerve fiber density was scored in representative sections at both fetal ages as absent (–), minimal (+), or dense (++) innervation.

Statistical Analysis

One-way repeated-measures ANOVA with the post hoc Holm-Bonferroni correction was used to analyze the different vasoconstrictor and vasodilator concentration-response curves in each age group with T_i as a dependent variable. Differences in concentration-response curves between the age groups were analyzed by two-way repeated-measures ANOVA with T_i as a dependent variable and group (0.7 vs. 0.9 gestation) as an independent variable.

The Student unpaired *t* test was used to determine differences in maximal effective contraction (C_{max} or $\%K_{max}$), maximal effective relaxation ($\%R_{max}$), and half maximal effective concentration, i.e., EC_{50} or $-\log(EC_{50})$ after testing for normal distribution. The calculation of the vascular sensitivity was based on the standard 4-parameter logistic equation using a self-written curve-fitting Excel routine, and was expressed as EC_{50} or $-\log(EC_{50})$, respectively. The paired Student *t* test was used to analyze subsequent vasodilator response at high NA and ET-1 concentrations (expressed as the difference in $\%K_{max}$).

The paired Student *t* test was used to analyze differences between the endothelium and media in each group regarding the media CSA and media-to-lumen ratio and the ratio of areas of positive IR. Group differences in the areas of positive IR in the endothelium and media and in the ratio of area of positive IR between endothelium and media were analyzed by the Student unpaired *t* test. Data are presented as means ± SEM. Significance was accepted if $p \leq 0.05$. SPSS (IBM SPSS Statistics, NY, USA) was used for statistical analysis.

Results

Age-Dependent Functional Changes

The resting T_i increased in tendency from 1.66 ± 0.16 mN at 0.7 gestation to 3.21 ± 0.65 mN at 0.9 gestation ($p = 0.078$). L_{100} increased from 310 ± 15 µm at 0.7 gestation to 515 ± 94 µm at 0.9 gestation ($p = 0.056$). Media CSA and media-to-lumen ratio did not change between 0.7 and 0.9 gestation.

Vasoconstrictor Responses

Non-receptor-mediated vasocontraction to K^+ was already present at 0.7 gestation and tended to increase at 0.9 gestation ($p = 0.074$). Receptor-mediated vasocontraction to NA and ET-1 was also present at 0.7 gestation and increased at 0.9 gestation. These developmental increases in vasoconstrictor responses were not accompanied by an increase in vascular sensitivity. ANGII as a single-dose application (10^{-5} M) and as a cumulative application ($10^{-12} - 10^{-6}$ M) failed to elicit a vasoconstrictor response at both gestational ages. The initial vasocontraction to NA and ET-1 were followed by vasodilator effects at high concentrations of NA ($10^{-5} - 10^{-4}$ M, 34.9 ± 12%, $p = 0.03$) and ET-1 (10^{-7} M, 19.5 ± 7%, $p = 0.04$) at 0.7 gestation, but not at 0.9 gestation (NA 9.7 ± 6%, $p = 0.18$; ET-1 3.2 ± 2%, $p = 0.18$; vasodilator effects are expressed as the difference in $\%K_{max}$ (Fig. 1).

Vasodilator Responses

Receptor-mediated, endothelium-dependent relaxation to ACh and PGE_2 was absent at 0.7 gestation, but clearly present at 0.9 gestation ($\%R_{max,\ ACh}$ $p = 0.005$, $\%R_{max\ PGE2}$ $p = 0.095$). In contrast, non-endothelium-dependent relaxation with the NO-donor SNP was present at 0.7 gestation and comparable to 0.9 gestation (Fig. 1).

Age-Dependent Structural Changes

Maturation of the Media

There were no differences in the media CSA and the media-to-lumen ratio at 0.7 gestation and at 0.9 gestation (Fig. 2). MHC-B and SM2 were detectable in the media at 0.7 gestation. The expression of the 2 myosin heavy-chain isoforms did not change in the period up to 0.9 gestation (Fig. 3).

Expression of Vasoactive Receptors in the Endothelium and Media

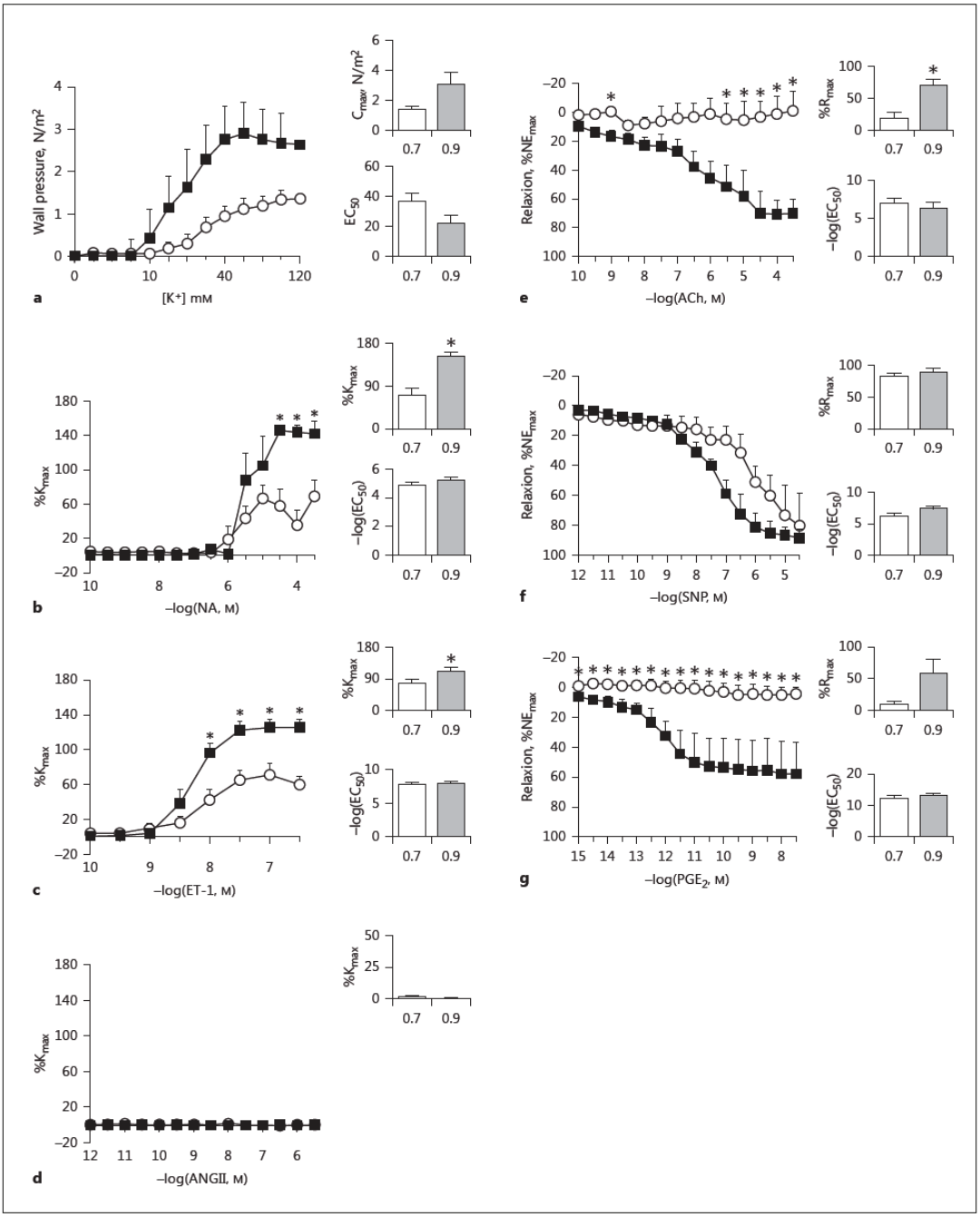
All the vasoactive receptors examined (α_{1A}, α_{2A}, and β_2 adrenoreceptors, $ET_{A/B}$, AChRM2/3, $EP_{2/4}$) were detectable in the media and endothelial cells at 0.7 gestation (Fig. 4, 5). Receptor expression was higher in the endothelium than in the media, except for that of α_{2A}, ET_A, and $EP_{2/4}$ (E/M ratio; Table 1). Receptor expression in the endothelium and media did not differ at 0.7 and 0.9 gestation except for that of AChRM3 ($p = 0.002$) and EP_4 ($p = 0.032$) which decreased in the media between 0.7 and 0.9 gestation (changes in E/M between 0.7 and 0.9 gestation, Table 1).

Expression of Vasoactive Enzymes in the Endothelium and Media

Both COX-2 and eNOS were detectable at 0.7 gestation and the expression of both enzymes was higher in the endothelium than in the media (E/M ratio; Table 1). Enzyme expression between the endothelium and the media did not differ between 0.7 and 0.9 gestation (changes in E/M between 0.7 and 0.9 gestation; Table 1).

Fig. 1. Mesenteric artery function to vasoconstrictors and vasodilators in fetal sheep. Values are means ± SEM for the concentration-response curves. Maximal contraction and sensitivity to potassium (K^+) (**a**), noradrenaline (NA) (**b**), endothelin-1 (ET-1) (**c**), angiotensin II (ANGII) (**d**). Maximal relaxation and sensitivity to acetylcholine (ACh) (**e**), sodium nitroprusside (SNP) (**f**) and prostaglandin E_2 (PGE_2) (**g**). Open circles, 0.7 gestation group; solid squares, 0.9 gestation group. * $p \leq 0.05$, significant differences between age groups (two-way ANOVA for repeated measurements + post hoc Tukey HSD correction). N/A calculation of $-\log(EC_{50})$ was not possible due to a lack of vasodilator response. $C_{max}/\%K_{max}$, maximal contraction; $\%R_{max}$, maximal relaxation ($EC_{50}/-\log(EC_{50})$), sensitivity.

(For figure see next page.)



1

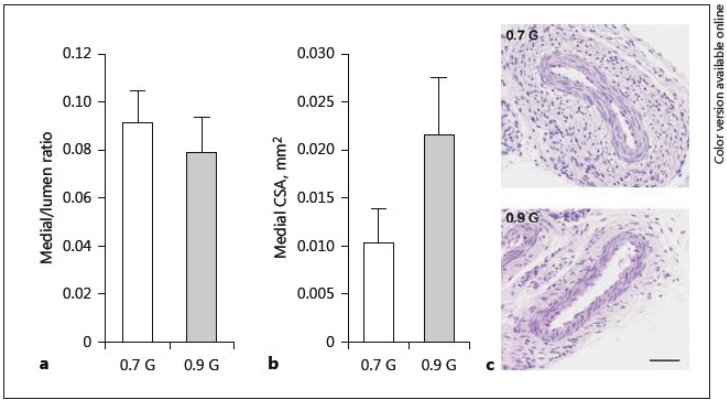


Fig. 2. The vessel wall in fetal mesenteric resistance arteries at 0.7 and 0.9 gestation. Mean ± SEM for the media-to-lumen ratio (**a**) and media cross-sectional area (CSA) (**b**). **c** Representative photomicrographs of mesenteric resistance arteries at 0.7 G and 0.9 G. HE. Scale bar, 100 µm. G, gestation.

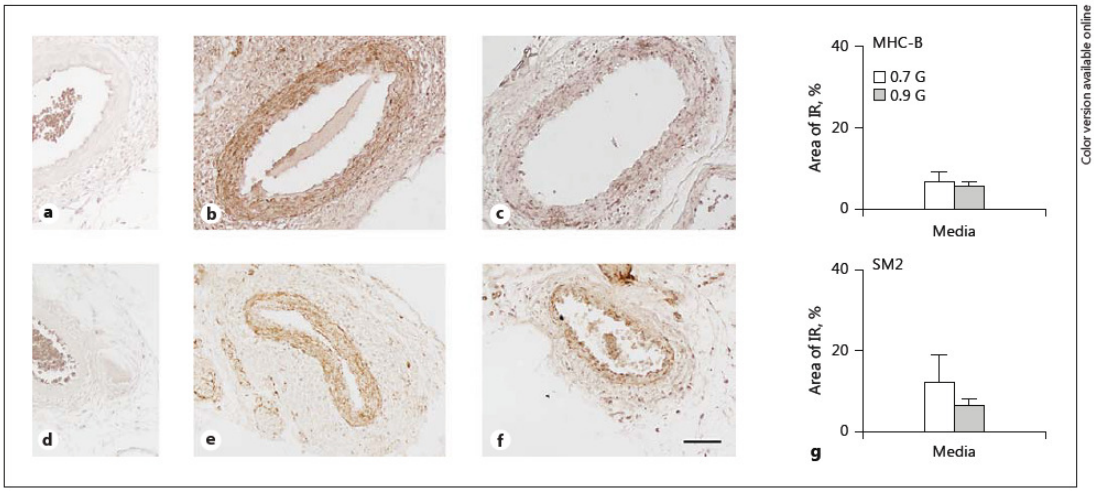


Fig. 3. Structural development of vascular smooth-muscle cells in fetal mesenteric resistance arteries. Representative photomicrographs of mesenteric resistance arteries: fetal (MHC-B; **b**, **c**) and adult (SM2; **e**, **f**) myosin heavy-chain isoforms and corresponding negative controls (**a**, **d**). Scale bar, 100 µm. **g** Image analysis of specific immunostaining in the media. Bars indicate the proportion of positive stained area in the media. Values are means ± SEM. IR, immunoreactivity; G, gestation.

Distribution of Sensory and Sympathetic Nerve Fibers

The adventitial mesenteric vascular layer contained more sensory CGRP-positive nerve fibers at 0.9 gestation than at 0.7 gestation (Fig. 6). In contrast, sympathetic innervation, represented by small granular TH-positive vesicles in the adventitia and media, was already present at 0.7 gestation and remained unchanged until 0.9 gestation.

Discussion

Although resting T_i increased between 0.7 and 0.9 gestation, major structural components of the vascular wall such as media mass (media CSA and media-to-lumen ratio) and the distribution of fetal and adult myosin heavy-chain isoforms did not change. Non-receptor-dependent vasocontraction to potassium and receptor-dependent

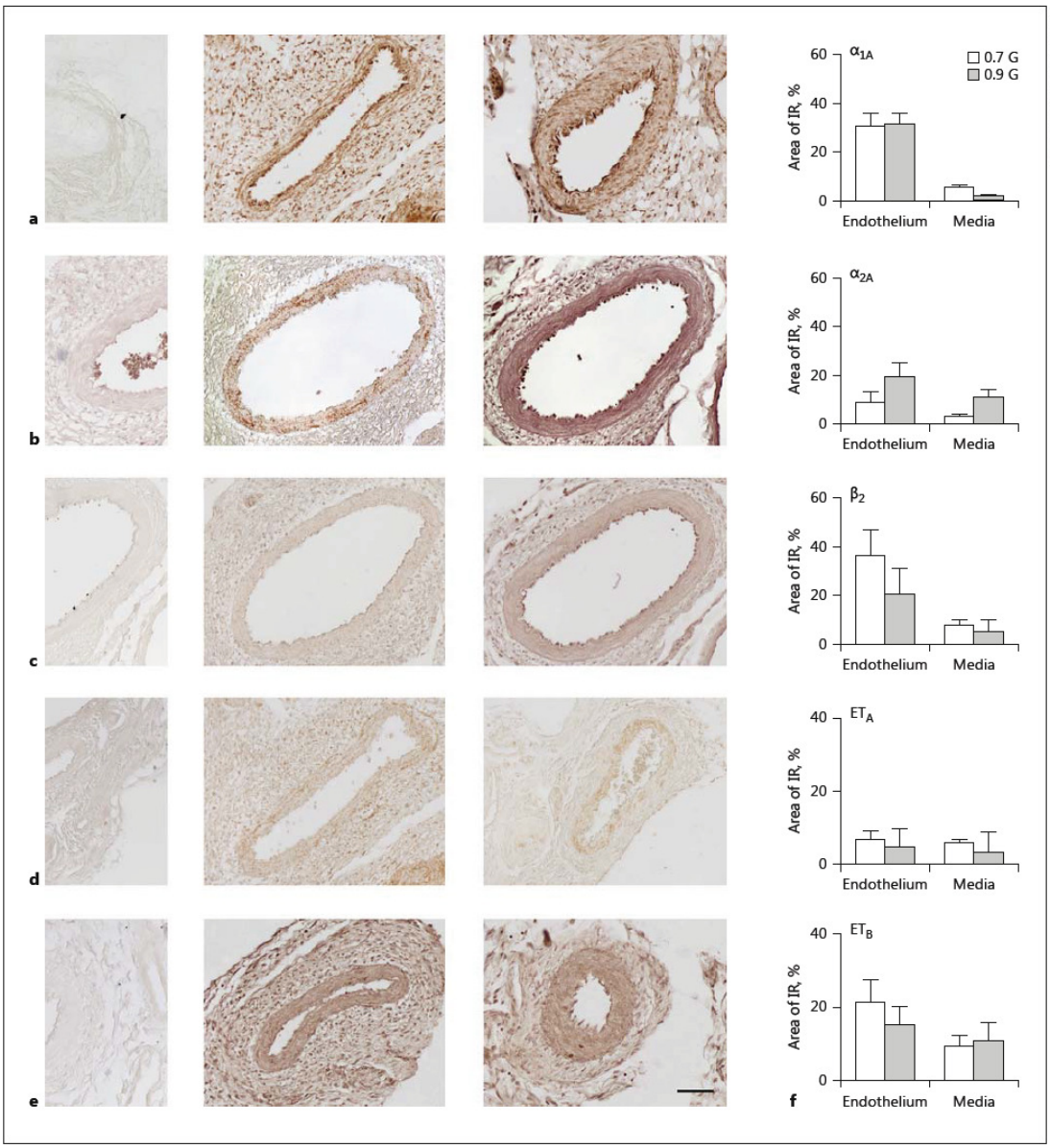
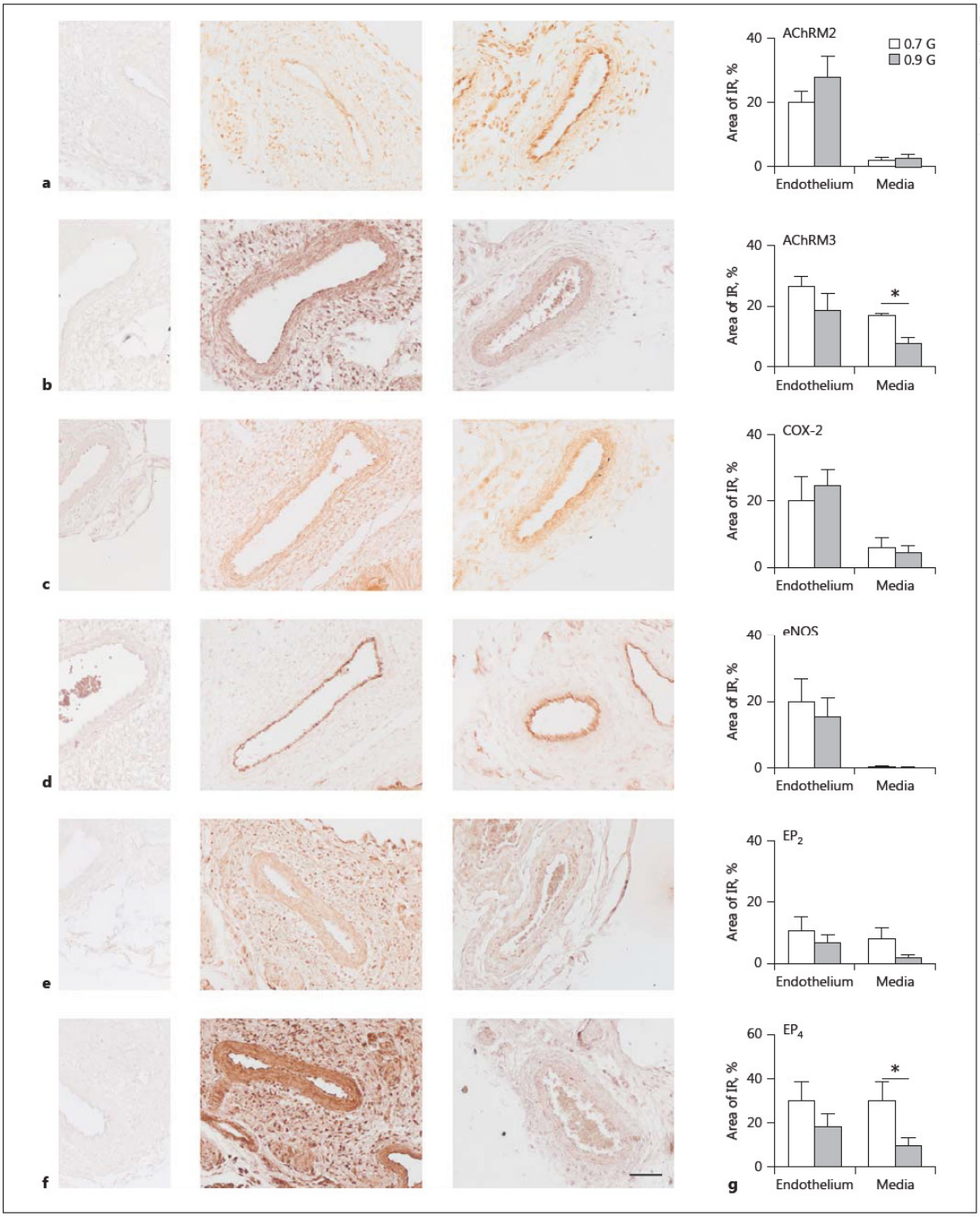


Fig. 4. Representative photomicrographs of the immunohistochemical distribution of vasoconstrictor components in fetal mesenteric resistance arteries in negative controls (left column), at 0.7 gestation (middle column), and at 0.9 gestation (right column). **a** α_{1A} adrenoreceptor. **b** α_{2A} adrenoreceptor. **c** β_2 adrenoreceptor. **d** ET_A. **e** ET_B. **f** Image analysis of specific immunostaining in the endothelium and media. Bars indicate the proportion of positive stained area. Values are means ± SEM. Scale bar, 100 μm. IR, immunoreactivity; G, gestation.



Color version available online

5

(For legend see next page.)

Table 1. Fetal mesenteric artery receptor expression using the ratio of area for immunoreactivity in the endothelium and media at 0.7 and 0.9 of ovine gestation

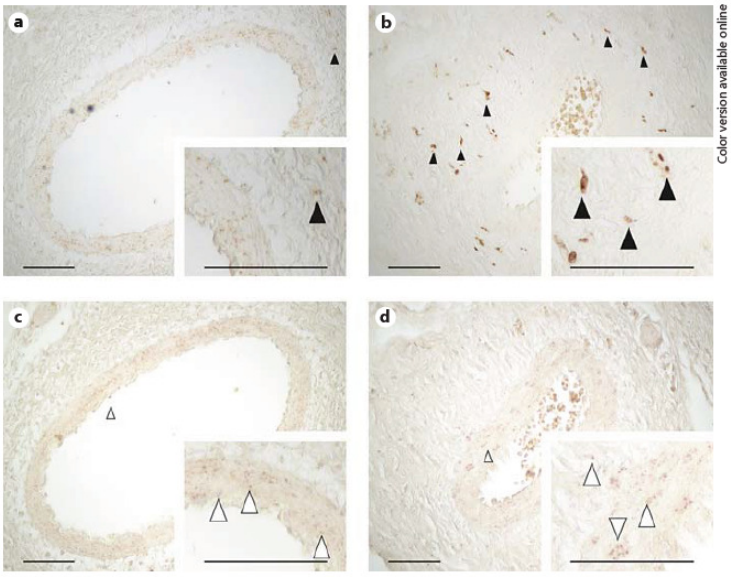
Marker	Ratio of area positive for IR								Changes in E/M ratio between 0.7 and 0.9 gestation *p* value
	at 0.7 gestation				at 0.9 gestation				
	E	M	E/M ratio, %	*p* value	E	M	E/M ratio, %	*p* value	
AChRM2	20.2±3.3	1.9±0.8	23.4±7.3	0.001	27.6±6.6	2.6±1.2	36.6±14.4	0.010	0.430
AChRM3	26.3±3.2	16.7±0.9	1.6±0.2	0.016	18.4±5.7	7.7±1.9	2.3±0.5	0.063	0.183
α_{1A}	30.6±5.2	5.1±1.5	9.5±2.6	0.002	31.7±4.2	1.9±0.6	219.2±202.2	0.001	0.347
α_{2A}	9.2±3,9	3.3±1.1	2.9±0.6	0.115	19.5±5.9	11.2±3.1	3.8±1.9	0.118	0.750
β_2	36.5±10.6	7.6±2.2	5.4± 0.8	0.029	20.7±10.3	5.3±4.7	14.5±7.9	0.128	0.337
COX-2	20.2±7.4	5.8±3.2	9.1±2.7	0.031	24.8±4.8	4.7±1.9	14.8±6.3	0.004	0.425
eNOS	20.4±6.8	0.2±0.2	6,022.9±3,685.2	0.031	15.5±6.0	0.1±0.1	485.3±277.0	0.051	0.208
ET_A	6.8±2.4	5.8±1.2	1.2±0.3	0.622	5.0±1.7	3.8±1.0	1.3±0.4	0.421	0.843
ET_B	21.3±6.2	9.5±2.7	3.8±1.5	0.045	15.2±4.8	10.6±5.0	4.4±2.3	0.007	0.830
EP_2	10.6±4.4	7.8±3.9	3.0±1.5	0.143	6.8±2.5	2.1±0.6	5.6±2.3	0.102	0.388
EP_4	30.8±8.2	30.8±8.3	1.0±0.1	1.00	18.3±6.0	9.8±3.3	5.4±3.4	0.335	0.274

Values are expressed as mean ± SEM. E, endothelium; M, media; AChRM2, muscarinic acetylcholine receptor M2; AChRM3, muscarinic acetylcholine receptor M3; α_{1A}, adrenoreceptor α_{1A}; α_{2A}, adrenoreceptor α_{2A}; β_2, adrenoreceptor β_2; COX-2, cycloogenase-2; eNOS, endothelial NO synthase; $ET_{A/B}$, endothelin-1 receptor type A/B; $EP_{2/4}$, prostaglandin E_2 receptor 2/4.

(NA, ET-1) vasocontraction was already present at 0.7 gestation but showed ongoing maturation at 0.9 gestation, indicating developmental regulation of media function. Sympathetic vascular innervation is established at 0.7 gestation and provides the basis for the autonomic regulation of mesenteric vascular tone and peripheral vascular resistance. Since the increase in medial contractility parallels the late gestational rise in fetal blood pressure [1, 13], it is highly likely that increases in peripheral mesenteric vasoconstrictive capacity contribute to this rise [1, 6, 14–16]. Receptor-dependent relaxation to ACh and PGE_2 began to mature after 0.7 gestation which was paralleled by an increase in CGRP-positive nerve fiber density. Thus, functional maturation of the endothelium and vasodilator adaptive sensory control takes place primarily in the last third of the period of gestation. In contrast, non-endothelium-dependent relaxation with NO was already maximal at 0.7 gestation, indicating the importance of the NO pathway in regulating vascular tone during early fetal life, e.g., via receptor-independent mechanisms [17]. Expression of corresponding vasoregulator receptors and enzymes as well as vascular sensitivity to the vasomediators did not show a developmental increase between 0.7 and 0.9 gestation. This suggests that maturation of vascular reactivity during the last third of the gestation period is due to the maturation of intracellular signaling pathways rather than to the maturation of receptor density or affinity.

The increase of the receptor-independent contractile capacity to potassium and of the resting T_i during the last third of gestation was not accompanied by changes in media mass (media CSA) or in the expression of myosin heavy-chain isoforms. In conduit vessels such as the aorta [18], middle cerebral [19] and carotid arteries [16], an increase in media mass and a higher expression of SM2 have been considered as major contributors to increased vascular contractile capacity during late gestation [10]. Future work is needed to determine whether these structural differences in media maturation between conduit and resistance arteries reflect region-specific or time course-specific functional demands within the fetal vascular tree. Since we did not find maturational changes in media mass and myosin heavy-chain distribution, other regulatory pathways need to be considered in order to

Fig. 5. Representative photomicrographs of the immunohistochemical distribution of vasodilator components in fetal mesenteric resistance arteries in negative controls (left column), at 0.7 gestation (middle column), and at 0.9 gestation (right column). **a** AChRM2. **b** Muscarinic AChRM3. **c** COX-2. **d** eNOS. **e** EP_2. **f** EP_4. **g** Image analysis of specific immunostaining in the endothelium and media. Bars indicate the proportion of positive stained area. Values are means ± SEM. * $p \leq 0.05$ for 0.7 G vs. 0.9 G. Scale bar, 100 µm. IR, immunoreactivity; G, gestation.



Color version available online

Fig. 6. Representative photomicrographs of the immunohistochemical distribution of calcitonin gene-related peptide at 0.7 (**a**) and 0.9 (**b**) gestation and tyrosine hydroxylase at 0.7 (**c**) and 0.9 (**d**) gestation. Scale bar, 100 μm. **Insets** Higher magnification: nerve fibers are labeled with arrowheads. Scale bar, 50 μm.

explain the developmental increase in receptor-independent vascular contractility. Ca^{2+}-dependent/-independent regulatory pathways of medial contractile response show major maturational changes in sheep during late gestation and early postnatal life [20–22]. Developmental increase in intracellular Ca^{2+} stores, density of L-type Ca^{2+} channels [20, 23] and rho-kinase activity [22] have been described in VSMCs in the cerebral, carotid, and aortic arteries in sheep. Developmental changes in these pathways in the resistance arteries are less well understood, but an increase in Ca^{2+} influx during late gestation has been observed in rabbit mesenteric VSMCs [24].

Apart from the maturation of the receptor-independent contractile capacity of the media and the sympathetic and sensory innervation, receptor-dependent vasocontraction to ET-1 and NA may contribute to the increase in resistance in the mesenteric vasculature. The receptor-dependent vasocontraction to ET-1 and NA, both major regulators of fetal vascular tone, is thought to participate in circulatory homeostasis during early and mid-gestation [25, 26]. In agreement with this, we observed vasoconstrictor responses to NA and ET-1 at 0.7 gestation; however, the magnitude and stability of the vascular response had continued to increase by 0.9 gestation. The gestational increase in vascular responsiveness to NA and ET-1 closely mirrors the developmental changes in ovine femoral resistance arteries [3], indicating a simultaneous functional maturation in the femoral and mesenteric vascular resistance arteries. The increase in vascular responsiveness to NA and ET-1 in our experiments parallels developmental changes in vivo that consist of an endogenous rise in NA and ET-1 plasma levels and enhanced systemic vascular and blood pressure responsiveness to NA and ET-1 [15, 27–34]. Notably, ANGII failed to elicit vasocontraction at either gestational age, reflecting the absence of vasocontraction-mediating type 1 ANGII receptors in the fetal mesenteric vasculature as previously described [35]. Thus, functional maturation of the NA and ET-1 pathways seems to be a major contributor to the physiological increase in peripheral vascular resistance and fetal systemic blood pressure during the last third of gestation [1, 6, 14–16].

The vasoconstrictor responses to ET-1 are mediated by the medial ET_A and ET_B whereas the endothelial ET_B mediates relaxation primarily by the release of prostacyclin [36, 37]. On the structural level, we found expression of both these receptors in the media and endothelium by 0.7 gestation, and this remained unchanged at 0.9 gestation. This finding underscores the potential role of receptor-dependent vasocontraction to ET-1 in circulatory ho-

meostasis in early and mid-gestation [25, 26]. Expression of ET_B was higher in the endothelium than in the media at both gestational ages, comparable to the human adult phenotype [38]. Thus, the expression of ET_A and ET_B precedes the functional maturation of ET-1-mediated vasocontraction, suggesting that its developmental increase depends on changes in the maturation of ET-1 downstream signaling rather than on the maturation of ET-1 receptor expression. Since vascular sensitivity was unchanged between 0.7 and 0.9 gestation, we can conclude that receptor expression and affinity are not involved in the maturational process.

Besides the vasoconstrictor effects of ET-1 at 0.7 gestation, we also observed a distinct vasodilator response at high ET-1 concentrations which was abolished at 0.9 gestation. We assume that the early vasodilator response is mediated by a parallel activation of endothelial ET_B at 0.7 gestation which is masked by a predominant vasoconstrictor ET_A activation during late gestation. In agreement with this assumption, a blockade of voltage-gated Ca^{2+}-channels prevents the ET-1-mediated vasocontraction and unmasks the vasodilator effect of high ET-1 dosages in adult arteries [39]. In the fetal vasculature, the density and capacity of voltage-gated Ca^{2+}-channels increased during the last third of gestation and is considered as a major contributor to ET_A-mediated vasocontraction [20, 40]. Therefore, maturation of the voltage-gated Ca^{2+}-channel may explain the different vasoconstrictor and dilator effects of ET-1 observed between 0.7 and 0.9 gestation.

The sympathetic innervation of peripheral resistance arteries is crucial for the maintenance of vascular tone and the adaptive control of peripheral vascular resistance. In accord with the vasoconstrictor response to NA, sympathetic vascular innervation was already established at 0.7 gestation, highlighting the interplay between sympathetic innervation and vascular NA responsiveness in regulating fetal mesenteric vascular tone. Given the involvement of TH in the production of catecholamines [41], the high density of TH-positive sympathetic nerve fibers further indicates that the splanchnic sympathetic nervous system is a leading source of NA in the systemic fetal circulation. Catecholamines primarily modulate vascular tone via adrenergic α_{1A}, α_2 (vasocontraction), and β_2 (relaxation) receptors. β_2 adrenoreceptors show a lower affinity for NA than α_{1A} and α_2 adrenoreceptors [42]. Notably, ontogenetic changes in the catecholamine pathway showed strong similarities to the ET-1 pathway. Thus, full expression of all 3 adrenoreceptor subtypes at 0.7 gestation preceded ongoing functional maturation of the vasoconstrictor NA response between 0.7 and 0.9 gestation. At both gestational ages, adrenoreceptor expression was higher in the endothelium than in the media, which resembles the adult mesenteric arterial expression pattern and is comparable to ET-1 [43, 44]. In contrast to our findings, Longo et al. [43] described a developmental decrease in VSMC α_{1A} adrenoreceptor expression in fetal to adult conduit cerebral arteries, providing evidence for the region-specific differences in vascular maturation and the importance of studying resistance vessels. Similar to ET-1, we found a vasodilator effect at high NA concentrations at 0.7 gestation, which was almost abolished at 0.9 gestation. The decreased vasodilator response was not accompanied by changes in α_{1A}, α_{2A}, and β_2 adrenoreceptor expression. Thus, analogous to our findings with ET-1, we conclude that functional changes in catecholamine-mediated vasoreactivity result from changes in downstream signaling rather than from changes in adrenoreceptor expression.

The endothelium-dependent vasodilator responses to ACh and PGE_2 developed between 0.7 and 0.9 gestation, reflecting functional endothelial maturation. Although endothelial AChRM2 and EP_2 expression was unchanged, AChRM3 and EP_4 expression in the media declined. The decrease in AChRM3 and EP_4 expression may reflect systemic changes in receptor VSMC type/subtype expression from early to late gestation, which has been described in the cholinergic, prostaglandin, and angiotensin systems [35, 45, 46]. The increase in endothelium-dependent vasorelaxation was accompanied by an increase in sensory CGRP-positive innervation, indicating the parallel maturation of endothelial and nerval vasodilator effector mechanisms. Nevertheless, the lack of increase in muscarinic and prostanoid receptor expression in the endothelium indicates that, as in the vasconstrictor pathways, major contributions to the developmental increase in vasodilator capacity result from changes in intracellular signaling cascades.

Downstream signaling of endothelium-dependent relaxation primarily involves the NO and prostaglandin pathways, with eNOS and COX-2 as key regulatory enzymes [47–49]. The vascular eNOS expression and the vasodilator NO response were fully established at 0.7 gestation, demonstrating the earlier maturation of the NO pathway compared to the endothelium-dependent vasodilator pathways. In agreement with our findings, Nishina et al. [50] demonstrated a profound response to SNP in the femoral resistance arteries already at 0.5 gestation. Later maturation of the endothelium-dependent, receptor-mediated vasodilator pathways further indicates that,

in earlier gestation, the NO pathway acts either via receptor-independent mechanisms (including shear stress and flow-mediated NO release) or via a receptor-dependent pathway other than muscarinic and prostanoid receptors [51–53]. In turn, since we did not observe changes in vascular eNOS and COX-2 expression between 0.7 and 0.9 gestation, it appears that other downstream signaling pathways, such as the endothelium-dependent membrane hyperpolarization factors or hydrogen sulfide, mediate the increase of endothelium-dependent receptor-mediated relaxation in the last third of gestation [54, 55]. The concept of the presence of key mediators other than NO and prostaglandins is further supported by the absence of effects of eNOS or COX-2 inhibition on the vasodilator response to ACh in fetal resistance and conduit arteries during the last third of gestation [56, 57].

In summary, the resting T_i and the vasoconstrictor and dilator capacity of mesenteric resistance arteries increase between 0.7 and 0.9 gestation. These changes are not attributed to the maturation of vascular structure or to an increase in vasoregulator receptor/enzyme density. Thus, changes in downstream signal cascades and Ca^{2+} sensitivity seem to be essential in mediating cardiovascular adaptation during late gestation. Our results emphasize that NO is an important vasodilator during early fetal life since the non-endothelium-dependent relaxation with NO was already maximal at 0.7 gestation. Several maturational aspects of the mesenteric resistance arteries are in contrast to the maturation of conduit arteries, underscoring the diverse developmental trajectories in different sectors of the vascular tree. A deeper knowledge of functional vascular maturation in different vascular beds will improve our understanding of the vulnerable periods for the development of persistent vascular dysfunction that predispose to cardiovascular disease in later life.

Acknowledgements

We thank Claudia Sommer and Ina Ingrisch for their great technical assistance and Nasim Kroegel for language editing. Furthermore, we thank Charles R. Rosenfeld who kindly provided antibodies for the smooth-muscle myosin heavy-chain isoforms (rabbit-anti-MHC-B and rabbit-anti-SM2).

Disclosure Statement

The authors have no potential conflicts of interest.

Funding

This work was supported by the European Union Seventh Framework Programme (FP7–HEALTH. 2011.2.2.2–2 BRAIN-AGE, grant agreement No: 279281).

References

1 Unno N, Wong CH, Jenkins SL, Wentworth RA, Ding XY, Li C, Robertson SS, Smotherman WP, Nathanielsz PW: Blood pressure and heart rate in the ovine fetus: ontogenic changes and effects of fetal adrenalectomy. Am J Physiol 1999;276:H248–H256.

2 Shinozuka N, Yen A, Nathanielsz PW: Increased myometrial contracture frequency at 96–140 days accelerates fetal cardiovascular maturation. Am J Physiol Heart Circ Physiol 2000;278:H41–H49.

3 Docherty CC, Kalmar-Nagy J, Engelen M, Nathanielsz PW: Development of fetal vascular responses to endothelin-1 and acetylcholine in the sheep. Am J Physiol Regul Integr Comp Physiol 2001;280:R554–R562.

4 Pearce WJ, Hull AD, Long DM, Longo LD: Developmental changes in ovine cerebral artery composition and reactivity. Am J Physiol 1991;261:R458–R465.

5 Su C, Bevan JA, Assali NS, Brinkman CR 3rd: Regional variation of lamb blood vessel responsiveness to vasoactive agents during fetal development. Circ Res 1977;41:844–848.

6 Hanson MA: The control of heart rate and blood pressure in the fetus: theoretical considerations; in Hanson MAS, John AD, Rodeck CH (eds): Fetus and Neonate Physiology and Clinical Applications. London, Cambridge University Press, 1993, vol 1, pp 1–19.

7 Barry JS, Anthony RV: The pregnant sheep as a model for human pregnancy. Theriogenology 2008;69:55–67.

8 Mulvany MJ, Halpern W: Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. Circ Res 1977;41:19–26.

9 Anwar MA, Schwab M, Poston L, Nathanielsz PW: Betamethasone-mediated vascular dysfunction and changes in hematological profile in the ovine fetus. Am J Physiol Heart Circ Physiol 1999;276:H1137–H1143.

10 Chern J, Kamm KE, Rosenfeld CR: Smooth-muscle myosin heavy-chain isoforms are developmentally-regulated in male fetal and neonatal sheep. Pediatr Res 1995;38:697–703.

11 Hart MN, Heistad DD, Brody MJ: Effect of chronic hypertension and sympathetic denervation on wall/lumen ratio of cerebral vessels. Hypertension 1980;2:419–423.

12 Schiffrin EL, Hayoz D: How to assess vascular remodelling in small and medium-sized muscular arteries in humans. J Hypertens 1997;15:571–584.

13 Matuszek MA, Gibson KJ, Lumbers ER, Simonetta G: Impact of cortisol on alpha-actin content in vascular smooth muscle cells of fetal sheep. Clin Exp Pharmacol P 2006;33:197–203.

14 Bevan JA, Bevan RD: Developmental influences on vascular structure and function. Ciba Found Symp 1981;83:94–107.

15 Frasch MG, Muller T, Wicher C, Weiss C, Lohle M, Schwab K, Schubert H, Nathanielsz PW, Witte OW, Schwab M: Fetal body weight and the development of the control of the cardiovascular system in fetal sheep. J Physiol 2007;579:893–907.

16 Hutanu C, Cox BE, DeSpain K, Liu XT, Rosenfeld CR: Vascular development in early ovine gestation: carotid smooth muscle function, phenotype, and biochemical markers. Am J Physiol Regul Integr Comp Physiol 2007;293:R323–R333.
17 Coumans ABC, Garnier Y, Supcun S, Jensen A, Hasaart THM, Berger R: The role of nitric oxide on fetal cardiovascular control during normoxia and acute hypoxia in 0.75 gestation sheep. J Soc Gynecol Invest 2003;10:275–282.
18 Arens WHJM, Rosenfeld CR, Kamm KE: Maturational differences between vascular and bladder smooth muscle during ovine development. Am J Physiol Regul Integr Comp Physiol 2000;278:R1305–R1313.
19 Goyal R, Henderson DA, Chu N, Longo LD: Ovine middle cerebral artery characterization and quantification of ultrastructure and other features: changes with development. Am J Physiol Regul Integr Comp Physiol 2012; 302:R433–R445.
20 Blood AB, Zhao Y, Long W, Zhang L, Longo LD: L-type Ca2+ channels in fetal and adult ovine cerebral arteries. Am J Physiol Regul Integr Comp Physiol 2002;282:R131–R138.
21 Goyal R, Creel KD, Chavis E, Smith GD, Longo LD, Wilson SM: Maturation of intracellular calcium homeostasis in sheep pulmonary arterial smooth muscle cells. Am J Physiol Lung Cell Mol Physiol 2008;295:L905–L914.
22 Goyal R, Mittal A, Chu N, Shi L, Zhang L, Longo LD: Maturation and the role of PKC-mediated contractility in ovine cerebral arteries. Am J Physiol Heart Circ Physiol 2009; 297:H2242–H2252.
23 Long W, Zhang L, Longo LD: Cerebral artery sarcoplasmic reticulum Ca(2+) stores and contractility: changes with development. Am J Physiol Regul Integr Comp Physiol 2000; 279:R860–R873.
24 Nakanishi T, Gu H, Abe K, Momma K: Developmental changes in the contractile system of the mesenteric small artery of rabbit. Pediatr Res 1997;41:65–71.
25 Nuwayhid B, Brinkman CR 3rd, Su C, Bevan JA, Assali NS: Development of autonomic control of fetal circulation. Am J Physiol 1975; 228:337–344.
26 Riley SC, Butt AR, Doughton BW, Li SX, Zheng SH, Findlay JK, Salamonsen LA: Endothelin in the ovine uterus during the oestrous cycle and early pregnancy. J Reprod Fertil 1994;100:451–459.
27 Cheung CY: Adrenal growth and ontogeny of adrenal substance P, enkephalins and catecholamines in the ovine fetus. J Dev Physiol 1989;12:77–84.
28 Cheung CY: Regulation of atrial-natriuretic-factor release by endothelin in ovine fetuses. Am J Physiol 1994;267:R380–R386.
29 Malamitsi-Puchner A, Antsaklis A, Economou E, Mesogitis S, Papantoniou N, Koutra N, Aravantinos D: Endothelin 1–21 plasma levels in fetuses at 18–24 weeks of gestation. J Perinat Med 1995;23:321–325.
30 Nakamura T, Kasai K, Konuma S, Emoto T, Banba N, Ishikawa M, Shimoda S: Immunoreactive endothelin concentrations in maternal and fetal blood. Life Sci 1990;46:1045–1050.
31 Nisell H, Hemsen A, Lunell NO, Wolff K, Lundberg MJ: Maternal and fetal levels of a novel polypeptide, endothelin: evidence for release during pregnancy and delivery. Gynecol Obstet Invest 1990;30:129–132.
32 Greenberg SG, Baker RS, Yang D, Clark KE: Effects of continuous infusion of endothelin-1 in pregnant sheep. Hypertension 1997; 30:1585–1590.
33 Jones OW 3rd, Abman SH: Systemic and pulmonary hemodynamic effects of big endothelin-1 and phosphoramidon in the ovine fetus. Am J Physiol 1994;266:R929–R955.
34 Jones CT, Ritchie JW: The cardiovascular effects of circulating catecholamines in fetal sheep. J Physiol 1978;285:381–393.
35 Cox BE, Rosenfeld CR: Ontogeny of vascular angiotensin II receptor subtype expression in ovine development. Pediatr Res 1999;45:414–424.
36 Haynes WG, Webb DJ: Contribution of endogenous generation of endothelin-1 to basal vascular tone. Lancet 1994;344:852–854.
37 Okawa T, Honda S, Sanpei M, Ishida T, Fujimori K, Sato A: Effects of nitric oxide and prostacyclin on hemodynamic response by big endothelin-1 in near term fetal sheep. J Perinat Med 2004;32:495–499.
38 Seo B, Oemar BS, Siebenmann R, von Segesser L, Luscher TF: Both ETA and ETB receptors mediate contraction to endothelin-1 in human blood vessels. Circulation 1994;89: 1203–1208.
39 Kiowski W, Luscher TF, Linder L, Buhler FR: Endothelin-1-induced vasoconstriction in humans. Reversal by calcium channel blockade but not by nitrovasodilators or endothelium-derived relaxing factor. Circulation 1991; 83:469–475.
40 Neylon CB: Vascular biology of endothelin signal transduction. Clin Exp Pharmacol Physiol 1999;26:149–153.
41 Thoenen H: Induction of tyrosine hydroxylase in peripheral and central adrenergic neurones by cold-exposure of rats. Nature 1970; 228:861–862.
42 Minneman KP, Pittman RN, Molinoff PB: Beta-adrenergic receptor subtypes: properties, distribution, and regulation. Annu Rev Neurosci 1981;4:419–461.
43 Longo LD, Ueno N, Zhao Y, Pearce WJ, Zhang L: Developmental changes in alpha 1-adrenergic receptors, IP3 responses, and NE-induced contraction in cerebral arteries. Am J Physiol 1996;271:H2313–H2319.
44 McGrath JC: Localization of α-adrenoceptors: JR Vane Medal Lecture. Br J Pharmacol 2015; 172:1179–1194.
45 Lee J, Banu SK, Nithy TK, Stanley JA, Arosh JA: Early pregnancy induced expression of prostaglandin E_2 receptors EP_2 and EP_4 in the ovine endometrium and regulated by interferon tau through multiple cell signaling pathways. Mol Cell Endocrinol 2012;348: 211–223.
46 Zhang L: Muscarinic receptors in developing rat colon. Eur J Pharmacol 1996;304:211–219.
47 Hristovska AM, Rasmussen LE, Hansen PB, Nielsen SS, Nusing RM, Narumiya S, Vanhoutte P, Skott O, Jensen BL: Prostaglandin E_2 induces vascular relaxation by E-prostanoid 4 receptor-mediated activation of endothelial nitric oxide synthase. Hypertension 2007;50: 525–530.
48 Spence VA, Khan F, Kennedy G, Abbot NC, Belch JJ: Acetylcholine-mediated vasodilatation in the microcirculation of patients with chronic fatigue syndrome. Prostaglandins Leukot Essent Fatty Acids 2004;70:403–407.
49 Simmons DL, Botting RM, Hla T: Cyclooxygenase isozymes: the biology of prostaglandin synthesis and inhibition. Pharmacol Rev 2004;56:387–437.
50 Nishina H, Green LR, McGarrigle HH, Noakes DE, Poston L, Hanson MA: Effect of nutritional restriction in early pregnancy on isolated femoral artery function in mid-gestation fetal sheep. J Physiol 2003;553:637–647.
51 Wieczorek KM, Brewer AS, Myatt L: Shear stress may stimulate release and action of nitric oxide in the human fetal-placental vasculature. Am J Obstet Gynecol 1995;173:708–713.
52 Li Y, Zheng J, Bird IM, Magness RR: Effects of pulsatile shear stress on nitric oxide production and endothelial cell nitric oxide synthase expression by ovine fetoplacental artery endothelial cells. Biol Reprod 2003;69:1053–1059.
53 Learmont JG, Poston L: Nitric oxide is involved in flow-induced dilation of isolated human small fetoplacental arteries. Am J Obstet Gynecol 1996;174:583–588.
54 Paul BD, Snyder SH: H_2S signalling through protein sulfhydration and beyond. Nat Rev Mol Cell Biol 2012;13:499–507.
55 Mathewson AM, Dunn WR: A comparison of responses to raised extracellular potassium and endothelium-derived hyperpolarizing factor (EDHF) in rat pressurised mesenteric arteries. PLoS One 2014;9:e111977.
56 Molnar J, Nijland MJM, Howe DC, Nathanielsz PW: Evidence for microvascular dysfunction after prenatal dexamethasone at 0.7, 0.75, and 0.8 gestation in sheep. Am J Physiol Regul Integr Comp Physiol 2002;283:R561–R567.
57 Török J, Gerová M: Developmental dynamics of endothelial and neurogenic control of canine thoracic aorta. Mech Ageing Dev 1997; 95:143–152.

4.2 Manuscript 2: Impact of chronic maternal psychosocial stress on the development of fetal blood pressure regulating arteries in sheep

Journal: Stress

Title: Impact of chronic maternal psychosocial stress on the development of fetal blood pressure regulating arteries in sheep

Julia J. Müller[1], Matthias Schwab[1], Florian Rakers[1], Charles R. Rosenfeld[2], Iwa Antonow-Schlorke[1], Peter W. Nathanielsz[3], Harald Schubert[4], Otto W. Witte[1], Sven Ruprecht[1]

[1] Hans Berger Department of Neurology, Jena University Hospital, Jena, Germany

[2] Division of Neonatal-Perinatal Medicine, University of Texas Southwestern Medical Center, Dallas, Texas, USA

[3] Wyoming Pregnancy and Life Course Health Center, University of Wyoming, Laramie Wyoming, USA

[4] Institute of Laboratory Animal Sciences and Welfare, Jena University Hospital, Jena, Germany

Running Title

Prenatal stress and vascular programming

Corresponding author:

Julia J. Müller, M. Sc.
Hans Berger Department of Neurology
Jena University Hospital
Am Klinikum 1
07747 Jena
Germany
Tel: +49-3641-9-396659
Email: Julia.Müller@med.uni-jena.de

Abstract

Background: Maternal psychosocial stress (MPS) predisposes for the development of arterial hypertension in later life. Knowledge of stress effects on maturation of blood pressure regulating arteries is important to identify mechanisms and vulnerable periods for development of vascular dysfunction in adulthood.

Methods: Pregnant ewes (term 150 days of gestation) underwent repeated isolation stress between 0.2-0.7 gestation (early stress) or between 0.7-0.9 gestation (late stress). Pregnant non-stressed ewes served as controls. We determined stress effects in fetal mesenteric resistance arteries and renal interlobular arteries at 0.7 gestation (early stress) and 0.9 gestation (30 days after early stress, and immediately after late stress) using wire-myography and immunohistochemistry.

Results: Early MPS resulted in an increase in endothelium-dependent vasoconstriction (endothlin-1) in mesenteric arteries and augmented vasodilatation (acetylcholine) in renal arteries at 0.7 gestation. These effects were replaced by decreased mesenteric noradrenergic vasoconstriction and blunted renal vasodilatation (NO, prostaglandin-E2) at 0.9 gestation. Late MPS did not affect vasoconstriction but increased endothelium-dependent Vasodilatation (acetylcholine) in mesenteric arteries and decreased NO-mediated Vasodilatation in renal arteries. MPS-induced changes in vasoreactivity were not reflected by the expression of the corresponding vasoregulator receptors/enzymes. Early and late MPS enhanced the expression of fetal smooth muscle myosin heavy chain isoforms in mesenteric but not renal arteries indicative for a delay in mesenteric smooth muscle cell maturation.

Conclusion: MPS changes the trajectory of blood pressure regulating arteries at the functional and structural level. Discordance between changes in vasoreactivity and vasoregulator receptor/enzyme expression suggests that alterations in vasoreactivity are due to programing of intracellular pathways.

Keywords: sheep, fetus, vascular development, prenatal stress

Introduction

10-15% of all pregnant women in high income countries and 10-41% in low and middle income countries report stress and stress-related mental disorders (56) (18). Beside its effects on maternal mental health, maternal psychosocial stress (MPS) interferes with fetal neurodevelopment and predisposes for mental and neurocognitive health disturbances diseases in the offspring (4) (3, 7, 20, 47, 52, 53).

However, MPS also predispose for the development of arterial hypertension and cardiovascular diseases in later life (17) (10).) The fetal cardiovascular system is particularly vulnerable to adverse environmental stimuli during pregnancy. In human studies, severe MPS particularly during early gestation predispose for cardiovascular diseases in adulthood (27, 38). Animal studies also described a strong linkage between fetal exposure to endogenous cortisol or synthetic glucocorticoids (GCs) and a persisting blood pressure increase in the offspring (13-15) (unser review). In agreement with the human studies, early and mid-gestation seem to be the most stress-vulnerable time windows for programming hypertension in the adult offspring of different species including rodents and ruminants (unser review).

Predisposition for arterial hypertension by MPS involves fetal programming of blood pressure regulating circuits including the hypothalamus-pituitary-adrenal (HPA) axis (30) and the autonomic nervous system (1, 26). MPS also interferes with renal development and alters activity of the renin-angiotensin-aldosterone-system (31, 36, 58). Animal data, derived from synthetic GC exposure, also imply direct developmental effects of stress hormones on the fetal vasculature, however the programming effects of MPS on the fetal vascular level are unknown. Moreover, the examination of different blood pressure regulating vascular beds at different time-points during pregnancy is mandatory to understand the entirety of MPS programming effects on the fetal vasculature.

Using the fetal sheep model we evaluate the effects of chronic isolation stress on the functional and structural maturation of a) the fetal mesenteric arteries as a major regulatory component of the peripheral vascular resistance system and b) the distal renal interlobular arteries, which contribute to the regulation of the renin-angiotensin-aldosterone-system by controlling renal cortical blood flow and glomerular hemodynamics (23, 43).

To determine vulnerable periods for the development of vascular dysfunction we analyzed the effects of MPS on vascular maturation during early and mid- gestation (0.2- 07 gestation) and late gestation (0.7- 0.9 gestation).

We used wire-myography, to assess vascular responsiveness to vasoconstrictors and -dilators that are crucial for the maintenance of basal vascular tone and adaptive vasoreactivity. Pathways involved in vascular tone regulation were characterized by immunohistochemical expression analysis of vasoregulator receptors and enzymes. Vascular smooth muscle cell characteristics were determined by evaluating the media mass and the expression of fetal and adult smooth muscle myosin heavy chain isoforms.

Methods

Animals

All procedures were approved by the Thuringia Animal Welfare Committee.

Stress protocol

Thirty-six Long-Wool Merino ewes were bred on a single occasion and randomly assigned to the experimental groups. To determine different stress-sensitive periods thirteen sheep followed a repeated maternal stress protocol between 0.2 (30±3 days term, 150 days,) and 0.7 (100±3 days) of gestation (referred to as early to mid-gestational stress) and eight ewes between 0.7 (100±3 days) 0.9 (130±3 days) of gestation (referred to as late gestational stress). Maternal stress was induced by a validated stress protocol including isolation of the pregnant sheep without any visual, tactile or auditory contact with flock-mates twice weekly for 3 hours (41).

To differentiate between acute and persistent effects of early to mid-gestational stress fetuses were examined at 0.7 of gestation (i.e. one day after the stress exposure, ewes n=7 wes) and at 0.9 of gestation (i.e. 30 days after the stress exposure, ewes n=6) Fetuses of the late gestational group were examined at 0.9 gestation (i.e. one day after the stress exposure, ewes n=8). Fetuses of unstressed ewes served as controls (0.7 gestation ewes n=7, 0.9 gestation ewes n=8)

Surgery

Only singleton and sibling fetuses were used for the experiments. All fetuses, were delivered by cesarean section at 0.7 gestation (100±3 days gestation, n=14) or at 0.9 gestation (130±3 days gestation, n = 22). In cases of sibling pregnancies, only one fetus was used for the experiments.

Anesthesia was induced by intramuscular injection of 1g ketamine (Ketamin-Hydrochlorid®, Pfizer, Berlin, Germany) and 0.2 mg/kg midazolam (Midazolam-Hameln®, Hameln Pharmaceuticals, Hameln, Germany) and maintained with 4% isoflurane (Isofluran – Actavis®, Actavis, Munich, Germany). Fetuses were killed by rapid exsanguination while still under general anesthesia.

Vessel function

Vessel preparation

The small intestine including the mesenteric root and one kidney were removed and stored in ice-cold physiological saline solution (PSS, pH 7.4: NaCl 119mM, KCl 4.7mM, $CaCl_2$ 2.5mM, $MgSO_4$ 1.17mM, $NaHCO_3$ 25mM, KH_2PO_4 1.18mM, EDTA 0.03mM, Glucose 5.5mM) for transport purposes for approximately 10 minutes. All chemicals were obtained from Sigma Aldrich, Steinheim, Germany. Third-branch mesenteric arteries and distal segments of the renal interlobular arteries were carefully dissected from surrounding tissue under the stereo microscope (~10-fold magnification) and transected into 2mm-long segments. Arterial segments (~ 400µm internal diameter) were threaded on 40 µm-diameter stainless-steel wires taking care not to damage the vessel structure or endothelium. Segments were transferred into a 5ml volume chamber and connected to an isometric force transducer (Multi Wire Myograph, Model 610M, DMT, Aarhus, Denmark). Arteries were equilibrated in PSS, warmed to 37°C, and gas-flushed with 95% O_2 and 5% CO_2. Data were digitally recorded using the WINDAQ data-acquisition system (WINDAQ, DATAQ Instruments, Akron, CA, USA).

Wire myography

The normalization was performed by distending the arterial segment stepwise and measuring internal circumference (IC_i) and wall tension T_i respectively. IC_{100} (i.e. internal circumference at the physiological transmural pressure of 13.3kPa (100mmHg) was caclulated by plotting wall tension against internal circumference and calculating the point of intersection with the isobar curve at 100mmHg using the Laplace relation. The normalized internal diameter l_{100} was calculated by dividing the normalized internal circumference by π (35). In a pretest experimental setting using a representive sample of five artery segments of both fetal ages vascular response to depolarizing potassium solution (125mM KPSS, equimolar substitution of NaCl with KCl in PSS) was obtained at different transmural pressure levels (0.5, 0.7 and 0.9x l_{100}) to determine optimal vascular responsivness (34). Vascular response to KPSS was maximal when normalized internal circumference was set to 0.9xIC_{100}, which is comparable to previous studies in rat and fetal ovine resistance arteries (12, 35). Therefore, l_{100} was set to 0.9xIC_{100} during the normalization procedure at the start of each experiment.

Resting wall tension was obtained after reaching a stable baseline level at least 30 min after normalization. Vascular integrity were ascertained using KPSS and 10^{-5} M noradrenaline (NA). Arteries showing an inadequate vasoconstrictor response to KPSS containing 10^{-5}M NA (i.e. not reaching the transmural pressure of 0.9x13.3kPa (100 mmHg)) were not considered for the experiments (2) Vasoconstrictor cumulative concentration-response curves were obtained for K^+ (1.25–20mM), NA (10^{-10} – $5x10^{-4}$M), endothelin-1 (ET-1; 10^{-10}–$5x10^{-7}$M) and angiotensin II (ANGII; 10^{-12} –$5x10^{-6}$M). Cumulative concentration-response curves for acetylcholine (Ach, 10^{-10} – $5x10^{-4}$M), prostaglandin- E_2 (PGE_2;10^{-15} – $5x10^{-8}$M) and the nitric oxide (NO)-donor sodium nitroprusside (SNP, 10^{-12} – $5x10^{-5}$M) were obtained after precontraction with $5x10^{-6}$M NA. Higher concentrations of the corresponding vasoactive agents were only added if the vascular tension showed a stable response plateau to the previous concentration. All vasoactive agents were dissolved in water.

Vasoconstrictor concentration-response curves to K^+ were normalized to internal circumference and vessel length (N/m²). Concentration-response curves to NA, ET-1 and ANGII were normalized to the maximal KPSS response (%K_{max}). Vasodilator response curves were normalized to stable precontraction levels (%R_{max}). The maximal vasoconstrictor response (C_{max} or %K_{max}) or maximal relaxation (%R_{max}) and the sensitivity (half maximal effective concentration, EC_{50}) were calculated for all agents. EC_{50} is given as -log(EC_{50}) for NA, ET-1, ACh, PGE_2, and SNP.

Data were analyzed by the same investigator blinded to group affiliation.

Mesenteric and renal artery morphology

Histological processing

We used immunohistochemistry since it allows localization of receptor and enzyme expression in the media and endothelium. Pretest histological examinations revealed, that immunofluorescence staining is not an appropriate staining method due to the auto-fluorescence of lamina propria in renal and mesenteric resistance arteries, which precludes quantitative analysis of epitope expression. Effects of maternal stress on structural vascular development was analyzed by the media cross sectional area and the media-to-lumen-ratio reflecting changes in media mass and relative organization of the media around the lumen as well as expression of fetal and adult smooth muscle myosin heavy chain

isoforms (fetal: MHC-B, adult: SM2) (8). Expression of endothelin-1 receptor type A and –B (ET_A and ET_B), adrenoreceptors α_{1A}, β_2, muscarinic acetylcholine receptors M2 and M3, (AChRM2, AChRM3) and the expression of vasoactive enzymes (endothelial NO synthase (eNOS); cyclooxygenase-2 (COX-2)) were determined in vascular smooth muscle cells (VSMCs) of the media and endothelial cells.

Multiple samples were taken from at least six fetal third-branch ovine mesenteric resistance arteries and distal segments of the renal interlobular arteries (~400µm internal diameter) of each experimental group. Arterial segments were fixed in neutral-buffered 4 % paraformaldehyde for at least one week and embedded in paraffin. Adjacent six-micrometer-sections were stained using polyclonal antibodies against AChRM2 (rabbit anti-AChRM2, 1:1000; SP4407P, Acris Antibodies, Herford, Germany), AChRM3 (rabbit-anti-AChRM3, 1:100, ab60981, Abcam, Cambridge, UK), eNOS (mouse anti-eNOS, 1:500, 610296, BD Laboratories, Heidelberg, Germany), adrenoreceptor α_{1A} (rabbit anti-adrenoreceptor α_{1A}, 1:200, SP 5126P, Acris Antibodies, Herford, Germany), adrenoreceptor β_2 (rabbit anti-adrenoreceptor β_2, 1:100, ABIN498188, Antibodies-online, Aachen, Germany), COX-2 (rabbit anti-COX-2, 1:600, LS-B5206, LifeSpan Bioscience, Eching, Germany,), ET_A (rabbit-anti ET_A, 1:250, G094-1, Assay Biotech, Sunnyvale, USA) and ET_B (rabbit-anti-ET_B, 1:700, SP4124P, Acris). After dewaxing, heat induced antigen retrieval was performed using sodium citrate buffer for staining AChRM2, α_{1A}-adrenoreceptor, eNOS and COX-2 (15 min in microwave at 750 W) and for detecting AChRM3, adrenoreceptor β_2 (pH 6.0, 20min at 80°C). Sections were first treated with 0.4% H_2O_2 for 20min to block tissue peroxidase activity, followed by 1mM $NaBH_4$ and incubated in 1.5% normal horse or goat serum (Vectastain® elite ABC KIT, Vector), respectively. The primary antibody was added overnight (4°C), followed by a secondary biotinylated antibody for 24 hours (1:200, 4°C; Vectastain® elite ABC KIT, Vector). Sections were incubated in a preformed avidin-horseradish peroxidase-complex (Vectastain® elite ABC KIT, Vector) for one hour at 37°C. Immunostaining was made visible by precipitation of diaminobenzidine (3.3'-diaminobenzidine tablets) as substrate for peroxidase (20min, 21°C). For negative controls, the primary antibodies were substituted by normal goat or horse serum. The concentration of each antibody was optimized by serial dilutions to achieve the optimal staining results. Finally, eight adjacent sections were stained with hematoxylin-eosin (HE) for morphometric analysis and to test the integrity of vascular

layers of mesenteric resistance arteries. When several arteries were present on the section, they were included in the analysis and values were averaged.

Image analysis of immunostaining

The positive immunoreactivity (IR) for AChRM2, AChRM3, eNOS, COX-2, α_{1A}, α_{2A} and β_2 adrenoreceptors, ET_A, ET_B, EP2 and EP4 was used to assess the respective protein expression in the media and endothelium on a semi-quantitative level. Light-microscopic images of the stained sections were digitized (x125 or x250) using a 3-charge-coupled device color video camera (Axiocam HR, Zeiss, Jena, Germany) under standardized conditions. In each section the whole area of the media and endothelium, as well as the positive immunostained area of these cell layers were assessed for each specific antibody using an image analysis program (Scion Image 6.21, NIH, USA). The percentage of area positive for IR was calculated for each layer. When several arteries were present on the section, they were included in the analysis and values were averaged. Artery wall and lumen areas were measured in HE stained sections, and the media cross sectional area and the media-to-lumen-ratio were calculated to determine arterial media mass and relative organization of the media around the lumen (24, 48). To address potential changes between medial (M) and endothelial (E) protein expression the ratio between the percentages of area positive for IR of both layers was calculated (E/M ratio) using at least six fetal third-branch ovine mesenteric resistance arteries and 6 distal renal interlobular artery segments per experimental group.

Evaluation of the histological sections was performed by the same investigator blinded to group affiliation.

Light-microscopic images of the immunohistochemically stained tissue were taken with a 3-charge-coupled device color video camera under standardized conditions at x125 or x250 magnification (Axiocam HR, Zeiss, Jena, Germany)

Statistical analysis

One-way repeated measurement ANOVA with *post hoc* Holm-Bonferroni correction was used to analyze the different vasoconstrictor and vasodilator concentration-response curves in each group with wall tension as dependent variable. Differences in concentration-response curves between the groups were analyzed by two-way repeated measurement ANOVA with wall tension as a dependent variable and group affiliation (control

0.7 gestation *vs*. early stress 0.7 gestation, control 0.9 gestation *vs*. early stress 0.9 gestation; control 0.9 gestation *vs*. late stress 0.9 gestation) as an independent variable. One-way ANOVA with Dunnets' or Tukeys' *post hoc* test was used to determine differences in maximal effective contraction (C_{max} or $\%K_{max}$), maximal effective relaxation ($\%R_{max}$) and half maximal effective concentration (EC_{50} or $-\log(EC_{50})$) between the groups after testing for normal distribution and equality of variances. The calculation of the vascular sensitivity was based on the standard four parameter logistic equation using a self-written curve-fitting excel routine and was expressed as EC_{50} or $-\log (EC_{50})$, respectively.

Paired Student's t-test was used to analyze differences in the media cross sectional area and media-to-lumen ratio and the ratio of areas of positive IR between endothelium and media in each group. Group differences in areas of positive IR in endothelium and media and in the ratio of area of positive IR between endothelium and media were analyzed by one-way ANOVA with Dunnets' or Tukeys' *post hoc* test after testing for normal distribution and equality of variances. Data are presented as means ± SEM. Significance was accepted if $p \leq 0.05$ and SPSS (IBM SPSS Statistics, NY, USA) was used for statistical analysis.

Results

All fetuses had a normal weight (control 0.7 gestation 1089±44g; control 0.9 gestation 3455±219g; early stress 0.7 gestation 1145 ±35g; early stress 0.9 gestation 3492±217g; late stress 0.9 gestation 3775±188g). Stress did not influence fetal weight or fetal kidney weight (control 0.7 gestation 11.42±0.73g; control 0.9 gestation 27.74±5.05; early stress 0.7 gestation 14.63±1.62; early stress 0.9 gestation 22.28±1.44; late stress 0.9 gestation 24.05±1.63).

A. Early stress-dependent changes in mesenteric arteries

Early stress (0.2 to 0.7 gestation) has no acute effects on resting wall tension at 0.7 gestation, but resulted in decrease in wall tension at 0.9 gestation (ANOVA: F=3.79, df= 2; $RT_{0.9G}$=3.21±0.65mN vs. $RT_{ES0.9G}$=1.56±0.38mN; p=0.039). Internal mesenteric vessel diameter (l_{100}) and maximal KPSS response were not influenced by early stress at 0.7 and 0.9 gestation.

Functional effects. Early stress did not acutely affect vascular response to NA but increased the vascular response to ET-1 at 0.7 gestation (ANOVA, F=6.25, df=1, control at 0.7 gestation vs. early stress at 0.7 gestation: $\%K_{maxET\text{-}1}$, p=0.029, figure XX). In contrast to the acute stress effects NA-mediated vasoconstriction was diminished at 0.9 gestation (ANOVA, F=10.09, df=2, p=0.001; control at 0.9 gestation vs. early stress at 0.9 gestation: $\%K_{maxNA}$; p=0.001, figure X). ET-1 mediated vasoconstriction did not differ between stressed and unstressed animals at 0.9 gestation. Vascular response to K^+ was not influenced by early stress at both gestational ages. Early stress had no effects on ACh, PGE2 and SNP-mediated Vasodilatation at 0.7 and 0.9 gestation (Figure 1).

Structural changes. Early stress had no acute (0.7 gestation) or long-term effects (0.9 gestation) on the media cross-sectional area and media-to-lumen-ratio of the mesenteric arteries. However, early stress increased expression of fetal MHC-B in mesenteric vascular smooth muscle cells at 0.7 gestation (ANOVA, F=5.49, df=4, p=0.003; control at 0.7 gestation vs. early stress at 0.7 gestation: p=0.07) and 0.9 gestation (p=0.05). SM2 expression was not affected by early stress at both gestational ages (Figure X).

Effects on receptor and enzyme expression. All examined vasoactive receptors (α_{1A} and β_2 adrenoreceptors, $ET_{A/B}$, and AChRM2/3) were detectable in stressed and unstressed

animal at both ages. Early stress had no acute effects on receptor expression at 0.7 gestation. Long-lasting effects included an increase in ET_A expression in the media (ANOVA, F=3.94, df=4, p=0.003; control at 0.9 gestation vs. early stress at 0.9 gestation: p=0.03) and decreased endothelial AChRM2 expression (p=0.05). The distribution of receptor expression (α_{1A} and β_2 adrenoreceptors, $ET_{A/B}$, and AChRM2/3) between endothelium and media (ratio of endothelial to media expression (E/M ratio) was not affected by early stress at both gestational ages. Expression of eNOS (endothelium) and COX-2 (endothelium and media) was detectable in the stressed groups at both ages. Expression of the vasoactive enzymes and the distribution between endothelium and media (E/M ratio, COX-2 only) was unaffected by early stress (0.7 and 0.9 gestation) (Figure X).

B. Early stress-dependent changes in renal arteries

Renal resting wall tension ($RT_{0.7G}$=2.52+0.38mN, $RT_{0.9G}$=2.32+0.41mN; p=1.00), maximal KPSS response ($KPSS_{0.7G}$=0.8+1.1mN/mm², $KPSS_{0.9G}$=9.5+1.4mN/mm²; p=0.94) and internal vessel diameter ($l_{100\ 0.7G}$=369.7+22.9µm, $l_{100\ 0.7G}$=445.5+52.0µm; p=0.84) were not influenced by early stress.

Functional effects. Early stress tended to increase ET-1 mediated response acutely at 0.7 gestation (ANOVA, F=3.68, df=1, p=0.6; control at 0.7 gestation vs. early stress at 0.7 gestation: $\%K_{maxET\text{-}1}$, p=0.08), without effects at 0.9 gestation (Figure x). K^+ and NA-mediated vasoconstriction were unaffected by early stress at both gestational ages. Early stress had no acute effects on SNP and PGE2 dependent Vasodilatation at 0.7 gestation but tended to increase ACh-mediated (ANOVA, F=4.38, df=1, control at 0.7 gestation vs. early stress at 0.7 gestation: $\%R_{maxACh}$, p=0.06) vasorelaxation. In contrast to acute effects sensitivity to SNP was diminished (ANOVA, F=4.99, df=2, p=0.017; control at 0.9 gestation vs. early stress at 0.9 gestation: $-logEC50_{SNP}$, p=0.04) and PGE_2 dependent Vasodilatation was increased at 0.9 gestation (ANOVA, F=5.51, df=2, p=0.014; control at 0.9 gestation vs. early stress at 0.9 gestation: $\%R_{max}$, p=0.05). ACh-dependent Vasodilatation showed no long-term stress effects.

Structural changes. Early stress (0.2 to 0.7 gestation) did not affect media cross-sectional area and media-to-lumen-ratio in renal arteries. Furthermore, early stress had no effects on MHC-B and SM2 expression in renal vascular smooth muscle cells.

Effects on receptor and enzyme expression. In renal arteries expression of vasoactive receptors (α_{1A}, β_2 adrenoreceptors, $ET_{A/B}$, AChRM2/3) were detectable in the endothelium and the media of stressed and unstressed animals at both ages (Figure X). Early stress had no acute effects on receptor expression at 0.7 gestation. Long-lasting effects included an increased expression of AChRM3 in the media (ANOVA, F=2.30, df=4, p=0.09; control at 0.9 gestation vs. early stress at 0.9 gestation: p=0.05) and a decreased expression of AChRM2 in endothelial cells (ANOVA, F=5.55, df=4, p=0.003; control at 0.9 gestation vs. early stress at 0.9 gestation: p=0.08) at 0.9 gestation. The ratio of endothelial to media expression (E/M ratio) of vasoactive receptors was not changed by fetal age or stress in renal arteries. Expression of vasoactive enzymes eNOS and COX-2 was detected in endothelial (eNOS, COX-2) and vascular smooth muscle cells (COX-2) in stressed and unstressed animals at 0.7 and 0.9 gestation. Early stress did not affect the expression of the vasoactive enzymes and the distribution between endothelium and media (E/M ratio, COX-2 only) at both gestational ages.

C. Late stress-dependent changes in mesenteric arteries

Late stress did not influence mesenteric resting wall tension or internal mesenteric vessel diameter (l_{100}) and maximal KPSS response at 0.9 gestation.

Functional effects. Vasoconstrictor response was not affected by late stress in mesenteric arteries. Late stress (0.7-0.9 gestation) increased sensitivity to ACh (ANOVA, F=6.35, df=2, p=0.009; control at 0.9 gestation vs. late stress at 0.9 gestation: $-\log EC_{50ACh}$, p=0.01), but not PGE2 or SNP in mesenteric arteries at 0.9 gestation.

Structural changes. Late stress (between 0.7 and 0.9 gestation) did not affect media cross-sectional area or media-to-lumen-ratio. However, late stress induced an increased expression of fetal MHC-B (ANOVA, F=5.49, df=4, p=0.003; control at 0.9 gestation vs. late stress at 0.9 gestation: MHC-B, p=0.02) in mesenteric vascular smooth muscle cells, without changes in SM2 expression at 0.9 gestation.

Effects on receptor and enzyme expression. All vasoactive receptors (α_{1A} and β_2 adrenoreceptors, $ET_{A/B}$, and AChRM2/3) were detectable in the media and in the endothelium in mesenteric arteries at 0.9 gestation. Late stress resulted in an increased ET_A expression solely in the media at 0.9 gestation (ANOVA, F=3.26, df=4, p=0.012; control at 0.9 gestation vs. late stress at 0.9 gestation: -logEC50, ETA p=0.06). Alpha-1A expression was

decreased by late stress solely within the endothelium (ANOVA, F=4.99, df=2, p=0.03; control at 0.9 gestation vs. late stress at 0.9 gestation: Alpha-1A p=0.09). Late stress did not affect the ratio of endothelial to media expression (E/M ratio). COX-2 was expressed in endothelial cells and in vascular smooth muscle cells and eNOS expression was limited on endothelial cells of stressed and unstressed animals at 0.9 gestation. Late stress had no effect on COX-2 or eNOS expression and on the distribution of both enzymes between endothelium and media (E/M ratio)

D. Late stress-dependent changes in renal arteries

Late stress did not affect renal resting wall tension or internal mesenteric vessel diameter (l_{100}) and maximal KPSS response at 0.9 gestation.

Functional effects. Vasoconstrictor response to NA, ET-1 and K+ was not affected by late stress. Late stress (0.7-0.9 gestation) resulted in a decreased sensitivity to SNP (ANOVA, F=4.99, df=2, p=0.017; control at 0.9 gestation vs. late stress at 0.9 gestation: -logEC50, p=0.02) but did not affect PGE_2 or ACh mediated vasorelaxation.

Structural changes. Late stress (between 0.7 and 0.9 gestation) had no effect on structural development (media cross-sectional area, media-to-lumen-ratio, MHC-B and SM2 expression).

Effects on receptor and enzyme expression. All vasoactive receptors (α_{1A}, $ET_{A/B}$, and AChRM2/3) were expressed in the media and in the endothelium in renal arteries at 0.9 gestation. Late stress did not influence vasoactive receptor expression in the media but induced an increase of endothelial AChRM2 expression (ANOVA, F=5.55, df=4, p=0.003; control at 0.9 gestation vs. late stress at 0.9 gestation: p=0.07). Endothelial expression of AChRM3, α_{1A} and ETA were not altered by late stress at 0.9 gestation. Late stress did not influence the distribution of both enzymes between endothelium and media (E/M ratio) of all examined vasoactive receptors. COX-2 was expressed in endothelial cells and in vascular smooth muscle cells and eNOS expression was limited on endothelial cells of stressed and unstressed animals at 0.9 gestation. Late stress had no effect on eNOS or COX-2 expression and distribution of both enzymes between endothelium and media (E/M ratio)

Discussion

Summary. The present study was designed to evaluate the effects of maternal psychosocial stress (MPS) on maturation of blood pressure regulating arteries at different time-points of gestation. MPS changed the trajectory of mesenteric and renal vascular development at the functional and structural level. Functional effects included vascular bed specific alterations of vasoconstricitve (ET1, and NA) and -dilative pathways (NO, ACh and PGE_2), which were dependent on time-point of stress exposure during gestation (early to mid-gestational vs. late gestational MPS) and unrelated to the vasoregulator receptor and enzyme expression. This suggests that MPS related changes in vasoreactivity are due to alterations of intracellular signaling pathways rather than to alterations of receptor density or affinity. On the structural level, early and late MPS delayed the development of smooth muscle cells in mesenteric but not renal vasculature potentially reflecting the different maturational status of both vascular beds during the prenatal stress exposure.

Effects of MPS during the time course of gestation need to be interpreted with respect to different maturational processes within the fetal vascular system. Renal vasoreactivity and sensory and sympathetic vascular innervation matures during early to mid-gestation paralleling the physiological kidney development (59). In contrast to the renal vasculature mesenteric vasoregulatory pathways and innervation primarily matures during mid- to late gestation (34). These developmental differences imply a different stress-susceptibility between the renal and mesenteric vascular bed during gestation. In agreement with this assumption, we observed a higher expression of fetal myosin isoforms (MHC-B) in the mesenteric but not renal arteries after MPS. This shift towards a fetal phenotype indicates a mesenteric specific delay in myocytal structural maturation and thus a higher stress vulnerability of the mesenteric vascular system during gestation.

However, MPS had heterogeneous effects on the maturation of several vasoregulatory pathways, which were detectable in both, the mesenteric and renal vascular bed. We found similar acute changes in vasoreactivity after early to mid-gestational MPS in renal and mesenteric arteries at 0.7 gestation. In detail, early MPS primarily enhanced ET-1-dependent vasoconstriction without affection of other vasoconstrictive pathways. Enhanced ET-1-dependent vasoconstriction at 0.7 gestation was not accompanied by changes in $ET_{A/B}$-receptor expression. Early MPS also induced an increase in endothelial

Vasodilatation to ACh in renal but not in mesenteric arteries which was also unreflected in AChRM2/3 receptor expression. Effects of early stress were also detectable at day 30 (0.9 gestation) after cessation of MPS which, however, differed from the acute effects observed at 0.7 gestation. At 0.9 gestation, early MPS primarily enhanced vasodilatory mechanisms potentially compensating the acute ET-1 mediated increase in vascular tone observed at 0.7 gestation. Vasodilatory effects differed between the vascular beds comprising alteration in PGE_2 and NO-dependent vasodilatation in renal arteries and a decrease in mesenteric resting wall tension and noradrenergic vasoreactivity without equivalent changes in receptor/enzymes expression. Late gestational MPS also influenced Vasodilatation in both renal and mesenteric arteries without affection of the vasoconstrictive pathways. However, vasodilatory effects differed between early and late gestational MPS and showed vascular bed specific characteristics. Whereas NO and PGE_2 mediated Vasodilatation was diminished in renal arteries, endothelium dependent Vasodilatation to ACh was increased in mesenteric arteries after late stress. Analogous to our findings after early gestational MPS we found no changes in the expression of the corresponding vasoregulator receptors and enzymes after late gestational MPS. We would therefore assume that MPS primarily affects the maturation of intracellular pathways involved in vasomotor control. Ca^{2+}-dependent and -independent regulatory pathways of medial contractile response show major maturational changes in sheep during late gestation and early postnatal life (5, 21, 22). Developmental increase in intracellular Ca^{2+} stores, density of L-type Ca^{2+} channels (5, 28) and rho-kinase activity (22) have also been described in vascular smooth muscle cells in cerebral, carotid and aortic arteries in sheep. To what extent MPS interferes with downstream signalling of the different vasoregulatory pathways should be clarified in further studies.

We have previously shown that acute MPS induces a reduction of uterine blood flow and shifts the fetal metabolism to an anaerobic state (40, 41). The reduction of uterine blood flow is prolonged after chronic MPS. Independent of the time point of stress exposure (i.e. early and late gestational MPS) chronic MPS increases fetal circulating baseline and stress-induced GC and norepinephrine concentrations with the greatest increase achieved in stressed early pregnant sheep. Thus, development of the HPA-axis and sympathetic-adrenal-medullary system is vulnerable to MPS during long periods of fetal life, whereas early gestation seems to be the most stress-vulnerable during gestation (16, 41).

Maturation of the fetal HPA axis and increase in circulating GC levels play an important role in fetal vascular reactivity and the physiologic development of the cardiovascular system (for review see: (19, 60). Glucocorticoids exert essential effects on vascular, morphology, growth, proliferation, and maintenance of vascular tone. GC effects are mainly mediated by the GC-receptor which is ubiquitary expressed in smooth muscle and endothelial cells. GC receptors are present since 0.2 of gestation and modulate transcriptional activation of several vasoregulatory pathways by suppression of vasodilator signalling and enhancing the effects of vasoconstrictor agents on endothelial cells and VSMCs in sheep (39, 60).

In the adult vasculature GCs sensitize and potentiate the vasoconstrictor effects of catecholamines and ET-1 on vascular smooth muscle cells and diminishes the vasodilator capacity by suppressing the production of vasodilators, including NO and prostaglandins (44). Effects of GCs on vascular function in the fetus has been characterized after administration of synthetic GCs in clinical dosages used to decrease neonatal mortality and morbidity by accelerating fetal lung maturation in women threatened by premature labor (6, 9). In agreement with our results after MPS, vascular effects of synthetic GCs depend on the time point of GC exposure during gestation and vary between the different vascular regions (Muller et al. 2018). However, the pattern of phenotypic changes substantially differs between the exposure to synthetic GCs and MPS. In contrast to our results after early MPS observed at 0.7 gestation, Roghair et al found no changes in mesenteric vasoreactivity after systemic administration of synthetic GCs during early gestation (0.2 gestation) in the late gestational fetus (0.8 gestation) (45). However, GC administration at 0.2 gestation resulted in diminished receptor dependent vasoconstriction in mesenteric (ET-1) and femoral (NE) arteries (50) in newborn lambs. Both vasoconstrictive pathways were not affected in the late gestational fetus (0.9 gestation) after exposure to early MPS. Late gestational (0.7-0.9 gestation) exposure to synthetic GCs predominantly affects ET-1 pathway characterized by enhanced ET-1-induced vasoconstriction in femoral resistance arteries (11, 32, 33). In contrast, ET-1 mediated vasoconstriction in mesenteric and renal arteries was not affected by late gestational MPS (0.7-0.9 gestation) in our study.

Considering these different phenotypic signatures of MPS and synthetic GC exposure, MPS mediated cortisol excess alone cannot fully explain the effects of MPS on vascular

development. Endogenous but not synthetic GC's are metabolized by two isoforms of the 11β-hydroxysteroid dehydrogenase (11β-HSD) in the vascular tissue. Whereas 11β-HSD1 converts cortisone to cortisol and thus amplifies local GC action 11β-HSD2 catalyze the interconversion of active GC (cortisol, corticosterone) to their inactive forms (cortisone, 11-dehydrocorticosterone) (49). A decrease in or inhibition of 11β-HSD 2 activity (i.e. the GC deactivating isozyme) leads to potentiation of vascular tone upon exposure to catecholamines and ET-1 (46, 57). Downregulation of 11β-HSD2 expression after MPS has been observed in the placenta and fetal liver (29, 37, 51). The highest concentration of these enzymes is located in resistance arteries compared to other regions of the vascular tree (57). Regional differences in 11β-HSD expression can also be observed during fetal development and might explain the vascular bed specific effects that we have observed after MPS (25, 54, 55). However, effects of MPS on fetal vascular expression of 11β-HSD2 remains unknown and should be addressed in following studies.

However, beside cortisol related mechanisms other potential mediators of the maternal fetal stress transfer including catecholamines, cytokines, serotonin/tryptophan, reactive-oxygen-species and fetal metabolic changes may contribute to the developmental changes in vascular system observed after MPS (42). We therefore propose that the effects of MPS on fetal vascular development are not a consequence of a single pathway but are mediated by multiple stress-transfer mechanisms acting together in a synergistic manner. A deeper knowledge of the multifactorial effects of maternal stress on vascular maturation is mandatory to improve our understanding of vulnerable periods for the development of persistent vascular dysfunction predisposing for cardiovascular disease in later life.

Acknowledgements

We thank Claudia Sommer and Ina Ingrisch for their great technical assistance and Nasim Kroegel for language editing. Furthermore, we thank Charles R. Rosenfeld who kindly provided antibodies for the smooth muscle myosin heavy chain isoforms (rabbit-anti-MHC-B and rabbit-anti-SM2).

Grants

This work was supported by the European Union Seventh Framework Programme (FP7—HEALTH. 2011.2.2.2-2 BRAINAGE, Grant Agreement no: 279281).

Disclosures

The authors have no potential conflicts of interest.

References

1. **Anwar MA, Saleh AI, Al Olabi R, Al Shehabi TS, and Eid AH.** Glucocorticoid-induced fetal origins of adult hypertension: Association with epigenetic events. *Vasc Pharmacol* 82: 41-50, 2016.
2. **Anwar MA, Schwab M, Poston L, and Nathanielsz PW.** Betamethasone-mediated vascular dysfunction and changes in hematological profile in the ovine fetus. *Am J Physiol-Heart C* 276: H1137-H1143, 1999.
3. **Bale TL, Baram TZ, Brown AS, Goldstein JM, Insel TR, McCarthy MM, Nemeroff CB, Reyes TM, Simerly RB, Susser ES, and Nestler EJ.** Early Life Programming and Neurodevelopmental Disorders. *Biol Psychiat* 68: 314-319, 2010.
4. **Beijers R, Buitelaar JK, and de Weerth C.** Mechanisms underlying the effects of prenatal psychosocial stress on child outcomes: beyond the HPA axis. *Eur Child Adoles Psy* 23: 943-956, 2014.
5. **Blood AB, Zhao Y, Long W, Zhang L, and Longo LD.** L-type Ca2+ channels in fetal and adult ovine cerebral arteries. *American journal of physiology Regulatory, integrative and comparative physiology* 282: R131-138, 2002.
6. **Cavalieri RL and Cohen WR.** Antenatal steroid therapy: Have we undervalued the risks? *J Matern-Fetal Neo M* 19: 265-269, 2006.
7. **Charil A, Laplante DP, Vaillancourt C, and King S.** Prenatal stress and brain development. *Brain Res Rev* 65: 56-79, 2010.
8. **Chern J, Kamm KE, and Rosenfeld CR.** Smooth-Muscle Myosin Heavy-Chain Isoforms Are Developmentally-Regulated in Male Fetal and Neonatal Sheep. *Pediatr Res* 38: 697-703, 1995.
9. **Crossley KJ, Morley CJ, Allison BJ, Davis PG, Polglase GR, Wallace MJ, Zahra VA, and Hooper SB.** Antenatal Corticosteroids Increase Fetal, But Not Postnatal, Pulmonary Blood Flow in Sheep. *Pediatr Res* 66: 283-288, 2009.
10. **de Vries A, Holmes MC, Heijnis A, Seier JV, Heerden J, Louw J, Wolfe-Coote S, Meaney MJ, Levitt NS, and Seckl JR.** Prenatal dexamethasone exposure induces changes in nonhuman primate offspring cardiometabolic and hypothalamic-pituitary-adrenal axis function. *J Clin Invest* 117: 1058-1067, 2007.
11. **Docherty CC, Kalmar-Nagy J, Engelen M, Koenen SV, Nijland M, Kuc RE, Davenport AP, and Nathanielsz PW.** Effect of in vivo fetal infusion of dexamethasone at 0.75 GA on fetal ovine resistance artery responses to ET-1. *Am J Physiol-Reg I* 281: R261-R268, 2001.
12. **Docherty CC, Kalmar-Nagy J, Engelen M, and Nathanielsz PW.** Development of fetal vascular responses to endothelin-1 and acetylcholine in the sheep. *American journal of physiology Regulatory, integrative and comparative physiology* 280: R554-562, 2001.
13. **Dodic M, Hantzis V, Duncan J, Rees S, Koukoulas I, Johnson K, Wintour EM, and Moritz K.** Programming effects of short prenatal exposure to cortisol. *Faseb J* 16: 1017-1026, 2002.
14. **Dodic M, McAlinden AT, Jefferies AJ, Wintour EM, Cock ML, May CN, Evans RG, and Moritz KM.** Differential effects of prenatal exposure to dexamethasone or cortisol on circulatory control mechanisms mediated by angiotensin II in the central nervous system of adult sheep. *J Physiol-London* 571: 651-660, 2006.
15. **Dodic M and Wintour EM.** Effects of Prolonged (48-H) Infusion of Cortisol on Blood-Pressure, Renal-Function and Fetal Fluids in the Immature Ovine Fetus. *Clin Exp Pharmacol P* 21: 971-980, 1994.
16. **Dreiling M, Schiffner R, Bischoff S, Rupprecht S, Kroegel N, Schubert H, Witte OW, Schwab M, and Rakers F.** Impact of chronic maternal stress during early gestation on maternal–fetal stress transfer and fetal stress sensitivity in sheep. *Stress*: 1-10, 2017.

17. **Entringer S.** Impact of stress and stress physiology during pregnancy on child metabolic function and obesity risk. *Curr Opin Clin Nutr Metab Care* 16: 320-327, 2013.
18. **Falah-Hassani K, Shiri R, and Dennis CL.** The prevalence of antenatal and postnatal co-morbid anxiety and depression: a meta-analysis. *Psychol Med*: 1-13, 2017.
19. **Fowden AL and Forhead AJ.** Glucocorticoids as regulatory signals during intrauterine development. *Exp Physiol* 100: 1477-1487, 2015.
20. **Glover V, O'Connor TG, and O'Donnell K.** Prenatal stress and the programming of the HPA axis. *Neurosci Biobehav Rev* 35: 17-22, 2010.
21. **Goyal R, Creel KD, Chavis E, Smith GD, Longo LD, and Wilson SM.** Maturation of intracellular calcium homeostasis in sheep pulmonary arterial smooth muscle cells. *American journal of physiology Lung cellular and molecular physiology* 295: L905-914, 2008.
22. **Goyal R, Mittal A, Chu N, Shi L, Zhang L, and Longo LD.** Maturation and the role of PKC-mediated contractility in ovine cerebral arteries. *American journal of physiology Heart and circulatory physiology* 297: H2242-2252, 2009.
23. **Hanson MA.** The control of heart rate and blood pressure in the fetus: theoretical considerations. In: *Fetus and Neonate- Physiology and clinical applications*, edited by Hanson MAS, John A. D.; Rodeck, Charles H. Department of Obstetrics and Gynaecology, University College and Middlesex School of Medicine, London: Cambridge University Press, 1993, p. 1-19.
24. **Hart MN, Heistad DD, and Brody MJ.** Effect of chronic hypertension and sympathetic denervation on wall/lumen ratio of cerebral vessels. *Hypertension* 2: 419-423, 1980.
25. **Hatakeyama H, Inaba S, Takeda R, and Miyamori I.** 11 beta-hydroxysteroid dehydrogenase in human vascular cells. *Kidney Int* 57: 1352-1357, 2000.
26. **Kapoor A, Dunn E, Kostaki A, Andrews MH, and Matthews SG.** Fetal programming of hypothalamo-pituitary-adrenal function: prenatal stress and glucocorticoids. *J Physiol-London* 572: 31-44, 2006.
27. **Laplante DP, Brunet A, Schmitz N, Ciampi A, and King S.** Project ice storm: Prenatal maternal stress affects cognitive and linguistic functioning in 51/2-year-old children. *J Am Acad Child Psy* 47: 1063-1072, 2008.
28. **Long W, Zhang L, and Longo LD.** Cerebral artery sarcoplasmic reticulum Ca(2+) stores and contractility: changes with development. *American journal of physiology Regulatory, integrative and comparative physiology* 279: R860-873, 2000.
29. **Maeyama H, Hirasawa T, Tahara Y, Obata C, Kasai H, Moriishi K, Mochizuki K, and Kubota T.** Maternal restraint stress during pregnancy in mice induces 11 beta-HSD1-associated metabolic changes in the livers of the offspring. *J Dev Orig Hlth Dis* 6: 105-114, 2015.
30. **Matthews SG and Phillips DI.** Transgenerational inheritance of stress pathology. *Exp Neurol* 233: 95-101, 2012.
31. **McMillen IC and Robinson JS.** Developmental origins of the metabolic syndrome: Prediction, plasticity, and programming. *Physiol Rev* 85: 571-633, 2005.
32. **Molnar J, Howe DC, Nijland MJM, and Nathanielsz PW.** Prenatal dexamethasone leads to both endothelial dysfunction and vasodilatory compensation in sheep. *J Physiol-London* 547: 61-66, 2003.
33. **Molnar J, Nijland MJM, Howe DC, and Nathanielsz PW.** Evidence for microvascular dysfunction after prenatal dexamethasone at 0.7, 0.75, and 0.8 gestation in sheep. *Am J Physiol-Reg I* 283: R561-R567, 2002.
34. **Muller JJ, Schwab M, Rosenfeld CR, Antonow-Schlorke I, Nathanielsz PW, Rakers F, Schubert H, Witte OW, and Rupprecht S.** Fetal Sheep Mesenteric Resistance Arteries: Functional and Structural Maturation. *J Vasc Res* 54: 259-271, 2017.
35. **Mulvany MJ and Halpern W.** Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. *Circulation research* 41: 19-26, 1977.
36. **Nuyt AM.** Mechanisms underlying developmental programming of elevated blood pressure and vascular dysfunction: evidence from human studies and experimental animal models. *Clin Sci* 114: 1-17, 2008.

37. **O'Donnell KJ, Jensen AB, Freeman L, Khalife N, O'Connor TG, and Glover V.** Maternal prenatal anxiety and downregulation of placental 11 beta-HSD2. *Psychoneuroendocrino* 37: 818-826, 2012.
38. **Painter RC, de Rooij SR, Bossuyt PM, Simmers TA, Osmond C, Barker DJ, Bleker OP, and Roseboom TJ.** Early onset of coronary artery disease after prenatal exposure to the Dutch famine. *Am J Clin Nutr* 84: 322-327, 2006.
39. **Peers A, Hantzis V, Dodic M, Koukoulas I, Gibson A, Baird R, Salemi R, and Wintour EM.** Functional glucocorticoid receptors in the mesonephros of the ovine fetus. *Kidney Int* 59: 425-433, 2001.
40. **Rakers F, Bischoff S, Schiffner R, Haase M, Rupprecht S, Kiehntopf M, Kuhn-Velten WN, Schubert H, Witte OW, Nijland MJ, Nathanielsz PW, and Schwab M.** Role of catecholamines in maternal-fetal stress transfer in sheep. *Am J Obstet Gynecol* 213, 2015.
41. **Rakers F, Frauendorf V, Rupprecht S, Schiffner R, Bischoff SJ, Kiehntopf M, Reinhold P, Witte OW, Schubert H, and Schwab M.** Effects of early-and late-gestational maternal stress and synthetic glucocorticoid on development of the fetal hypothalamus–pituitary–adrenal axis in sheep. *Stress* 16: 122-129, 2013.
42. **Rakers FR, Sven; Dreiling, Michelle; Bergmeier, Christoph; Witte, Otto W.; Schwab, Matthias.** Transfer of Maternal Psychosocial Stress to the Fetus. *Neuroscience & Biobehavioral Reviews* X: X, 2017.
43. **Robillard JE and Nakamura KT.** Neurohormonal regulation of renal function during development. *Am J Physiol* 254: F771-779, 1988.
44. **Rogers KM, Bonar CA, Estrella JL, and Yang SM.** Inhibitory effect of glucocorticoid on coronary artery endothelial function. *Am J Physiol-Heart C* 283: H1922-H1928, 2002.
45. **Roghair RD, Lamb FS, Miller FJ, Scholz TD, and Segar JL.** Early gestation dexamethasone programs enhanced postnatal ovine coronary artery vascular reactivity. *Am J Physiol-Reg I* 288: R46-R53, 2005.
46. **Ruschitzka F, Quaschning T, Noll G, deGottardi A, Rossier MF, Enseleit F, Hurlimann D, Luscher TF, and Shaw SG.** Endothelin 1 type A receptor antagonism prevents vascular dysfunction and hypertension induced by 11 beta-hydroxysteroid dehydrogenase inhibition - Role of nitric oxide. *Circulation* 103: 3129-3135, 2001.
47. **Sandman CA, Davis EP, Buss C, and Glynn LM.** Exposure to prenatal psychobiological stress exerts programming influences on the mother and her fetus. *Neuroendocrinology* 95: 7-21, 2012.
48. **Schiffrin EL and Hayoz D.** How to assess vascular remodelling in small and medium-sized muscular arteries in humans. *J Hypertens* 15: 571-584, 1997.
49. **Seckl JR and Walker BR.** Minireview: 11 beta-hydroxysteroid dehydrogenase type 1 - A tissue-specific amplifier of glucocorticoid action. *Endocrinology* 142: 1371-1376, 2001.
50. **Segar JL, Roghair RD, Segar EM, Bailey MC, Scholz TD, and Lamb FS.** Early gestation dexamethasone alters baroreflex and vascular responses in newborn lambs before hypertension. *Am J Physiol-Reg I* 291: R481-R488, 2006.
51. **Seth S, Lewis AJ, Saffery R, Lappas M, and Galbally M.** Maternal Prenatal Mental Health and Placental 11 beta-HSD2 Gene Expression: Initial Findings from the Mercy Pregnancy and Emotional Wellbeing Study. *Int J Mol Sci* 16: 27482-27496, 2015.
52. **Swanson JD and Wadhwa PM.** Developmental origins of child mental health disorders. *J Child Psychol Psychiatry* 49: 1009-1019, 2008.
53. **Talge NM, Neal C, Glover V, Early Stress TR, Prevention Science Network F, Neonatal Experience on C, and Adolescent Mental H.** Antenatal maternal stress and long-term effects on child neurodevelopment: how and why? *J Child Psychol Psychiatry* 48: 245-261, 2007.
54. **Thompson A, Han VKM, and Yang K.** Differential expression of 11 beta-hydroxysteroid dehydrogenase types 1 and 2 mRNA and glucocorticoid receptor protein during mouse embryonic development. *J Steroid Biochem* 88: 367-375, 2004.

55. **Ullian ME.** The role of corticosteroids in the regulation of vascular tone. *Cardiovasc Res* 41: 55-64, 1999.
56. **UNFPA W.** Maternal mental health and child health and development in low and middle income countries. *Geneva, Switzerland: Report of UNFPA and WHO*, 2008.
57. **Walker BR, Yau JL, Brett LP, Seckl JR, Monder C, Williams BC, and Edwards CRW.** 11-Beta-Hydroxysteroid Dehydrogenase in Vascular Smooth-Muscle and Heart - Implications for Cardiovascular-Responses to Glucocorticoids. *Endocrinology* 129: 3305-3312, 1991.
58. **Wintour EM, Johnson K, Koukoulas I, Moritz K, Tersteeg M, and Dodic M.** Programming the cardiovascular system, kidney and the brain - A review. *Placenta* 24: S65-S71, 2003.
59. **Wintour EM and Moritz KM.** Comparative aspects of fetal renal development. *Equine Vet J Suppl*: 51-58, 1997.
60. **Yang S and Zhang L.** Glucocorticoids and vascular reactivity. *Current vascular pharmacology* 2: 1-12, 2004.

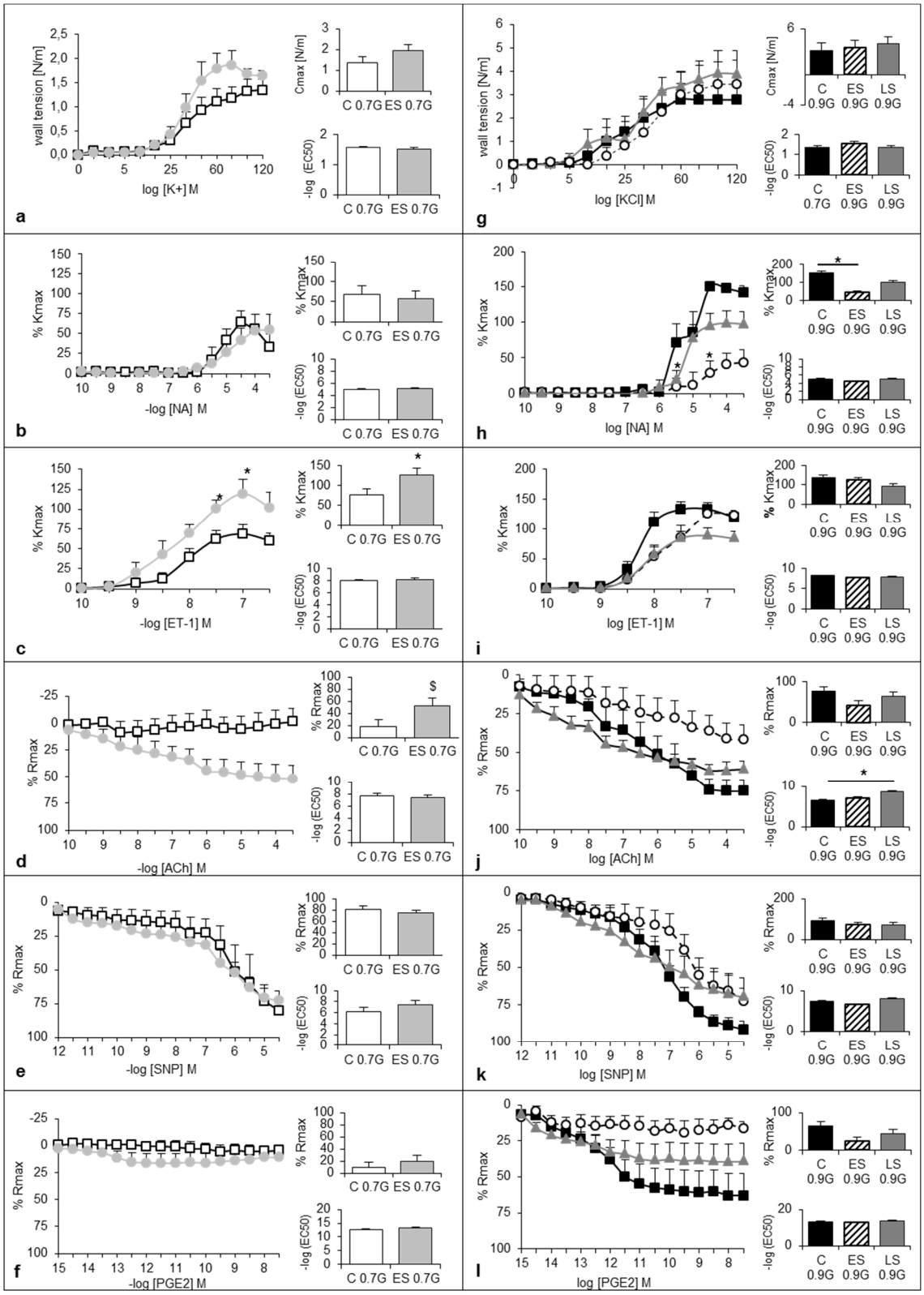


Fig. 1 Stress effects on dosis response curves of mesenteric resistance arteries at 0.7 (a-f) and 0.9 gestation (g-l). K^+ potassium, NA noradrenalin, ACh Acetylcholine, SNP sodium nitroprusside, C 0.7 G control at 0.7 gestation, ES 0.7G early stress at 0.7 gestation, C 0.9G control at 0.9 gestation, ES 0.9G early stress at 0.9 gestation, LS 0.9G late stress at 0.0 gestation. Data are mean ± SEM. *P < 0.05, $ P<0.1.

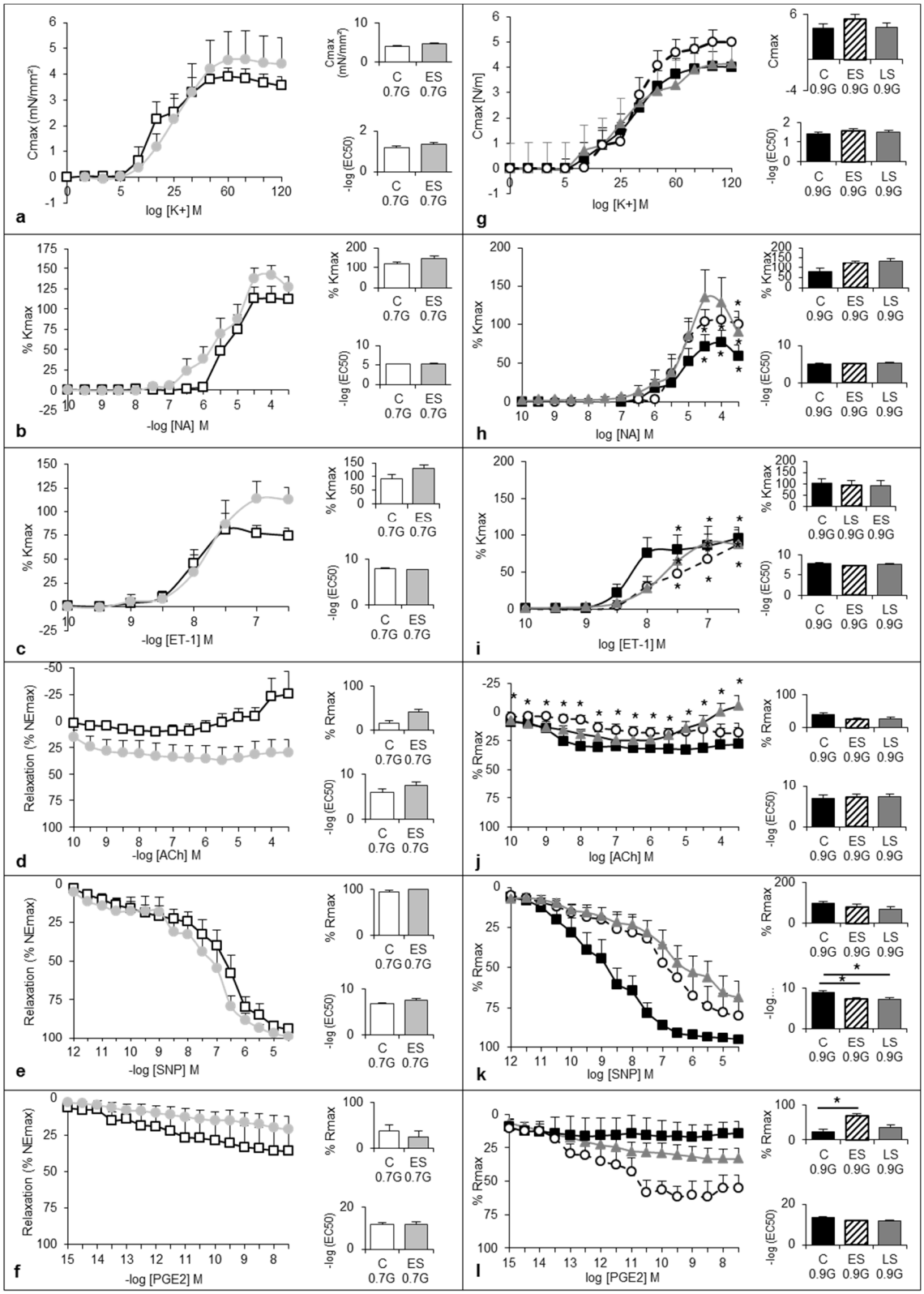


Fig. 2 Stress effects on dosis response curves of renal arteries at 0.7 (a-f) and 0.9 gestation (g-l). K^+ potassium, NA noradrenalin, ACh Acetylcholine, SNP sodium nitroprusside, C 0.7 G control at 0.7 gestation, ES 0.7G early stress at 0.7 gestation, C 0.9G control at 0.9 gestation ,ES 0.9G early stress at 0.9 gestation, LS 0.9G late stress at 0.0 gestation. Data are mean ± SEM. *P < 0.05, $ P<0.1.

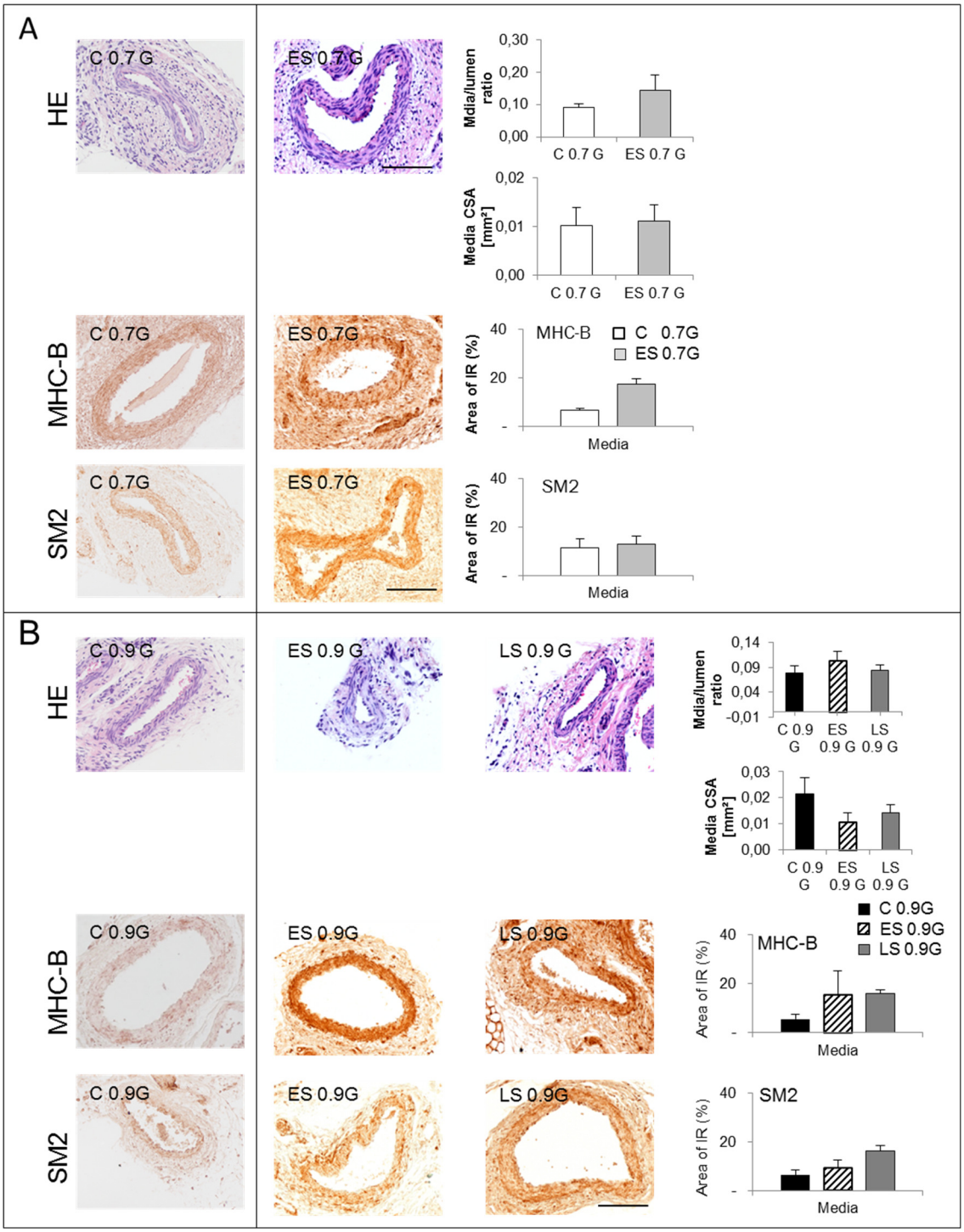


Fig 3. Morphologic effects of early stress and late stress on mesenteric arteries. A. Stress effects at 0.7 gestation B. stress effects on 0.9 gestation. C 0.7 G: controls at 0.7 gestation. ES 0.7 G: early stress at 0.7 gestation. C 0.9 G: controls at 0.9 gestation. ES 0.9 G: early stress at 0.7 gestation. LS 0.9 G: late stress at 0.9 gestation. Area of IR (%) Area of imunoreactivity

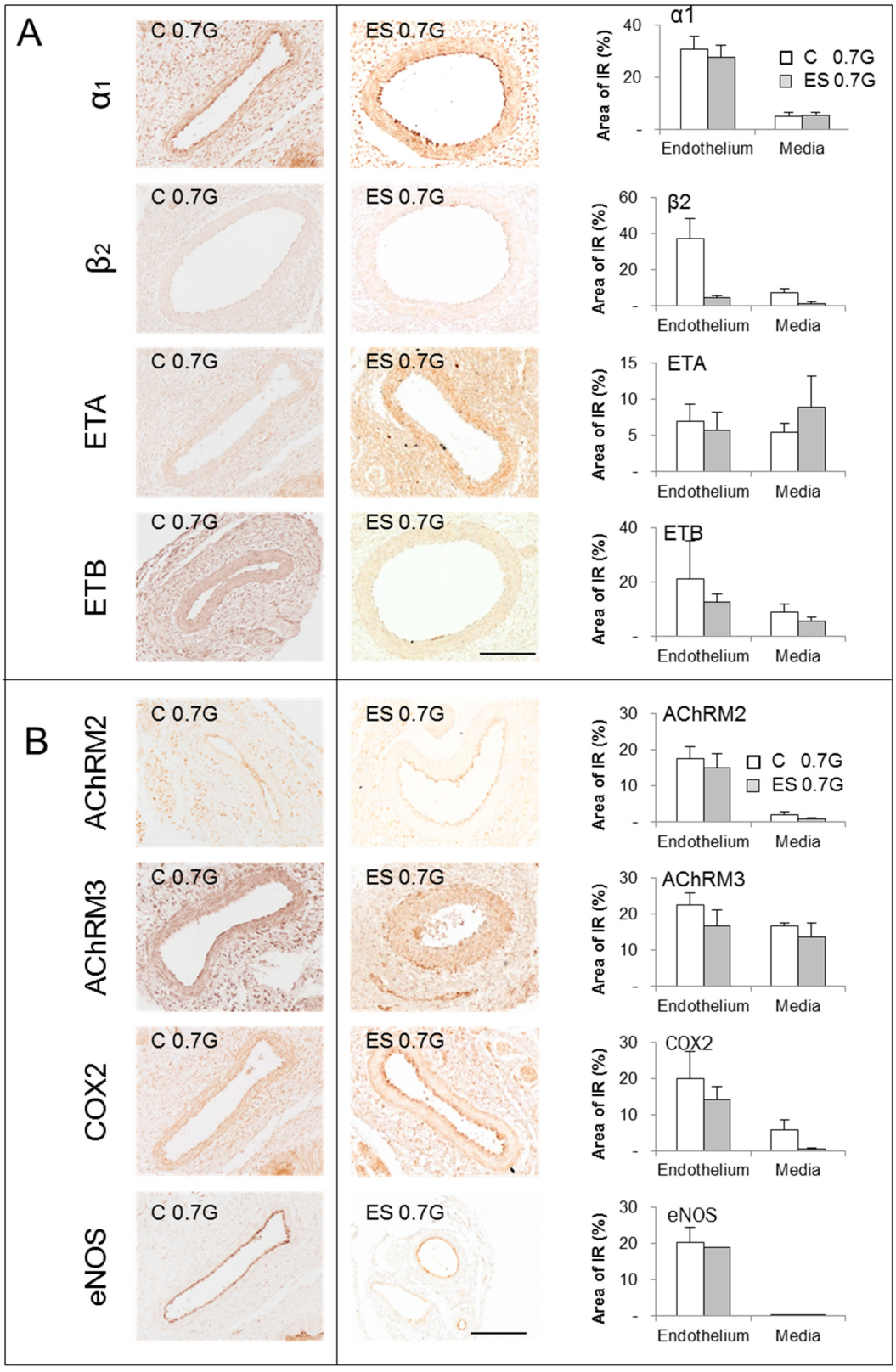


Fig.4 Vascular effects of early stress on mesenteric arteries at 0.7 gestation. A. effects on vasoconstrictive acting receptors B. effects on vasodilative acting receptors and enzymes. C 0.7 G: controls at 0.7 gestation. ES 0.7 G: early stress at 0.7 gestation. Area of IR (%) Area of imunoreactivity

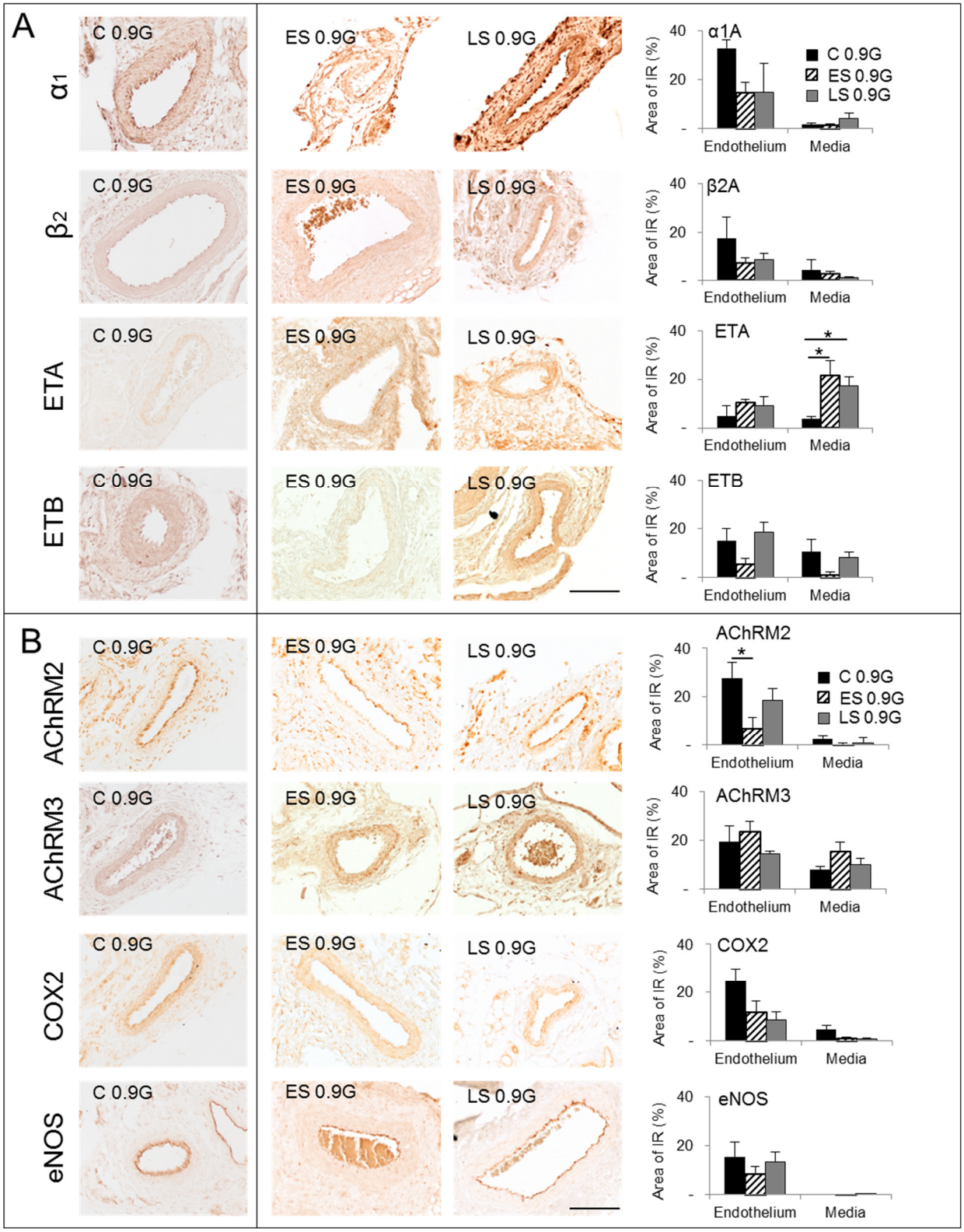


Fig. 5 Vascular effects of early stress on mesenteric arteries at 0.9 gestation. A. effects on vasoconstrictive acting receptors B. effects on vasodilative acting receptors and enzymes. C 0.9 G: controls at 0.9 gestation. ES 0.9 G: early stress at 0.7 gestation. LS 0.9 G: late stress at 0.9 gestation. Area of IR (%) Area of imunoreactivity

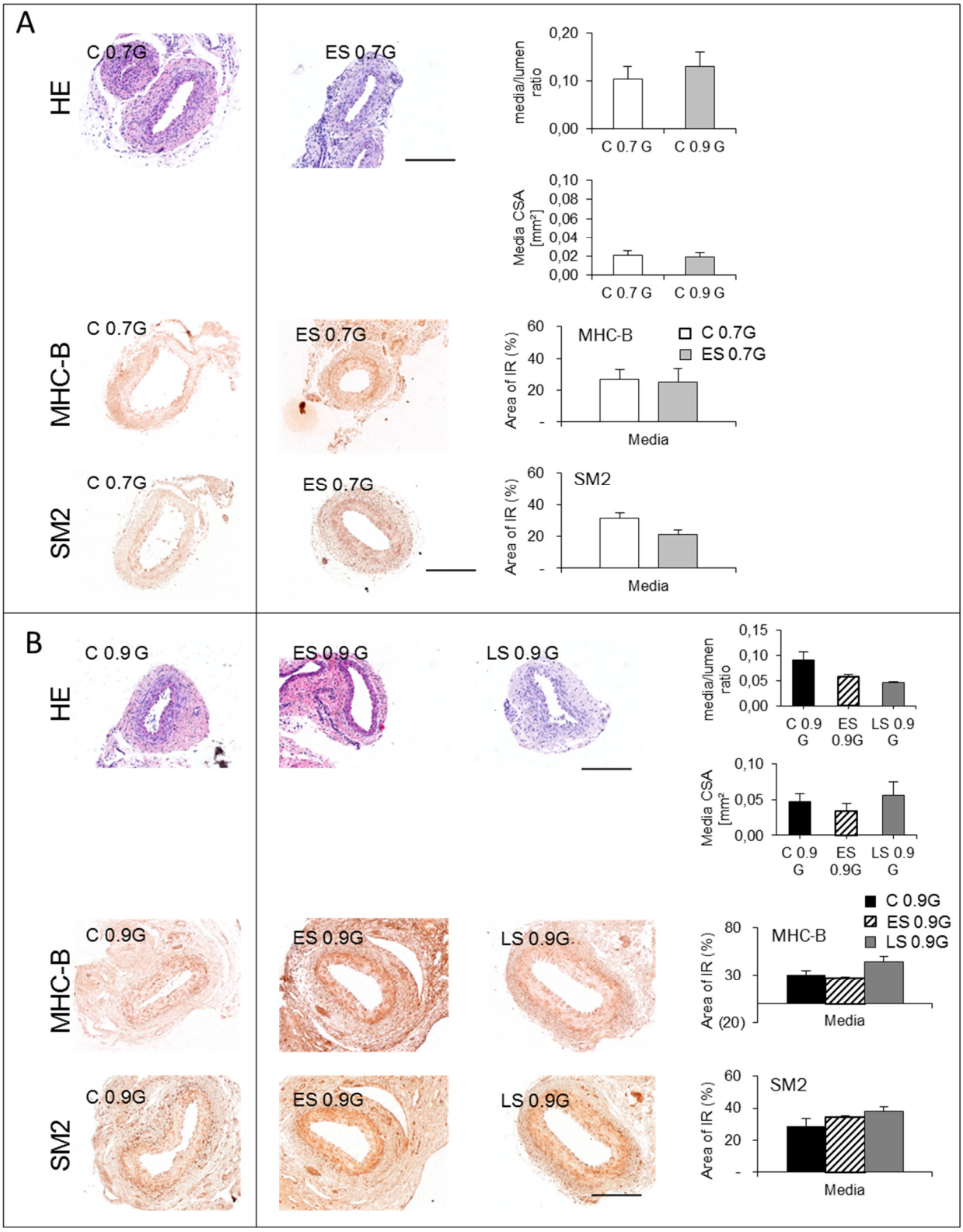


Fig 6. Morphologic effects of early stress and late stress on renal arteries. A. Stress effects at 0.7 gestation B. stress effects on 0.9 gestation. C 0.7 G: controls at 0.7 gestation. ES 0.7 G: early stress at 0.7 gestation. C 0.9 G: controls at 0.9 gestation. ES 0.9 G: early stress at 0.7 gestation. LS 0.9 G: late stress at 0.9 gestation. Area of IR (%) Area of imunoreactivity

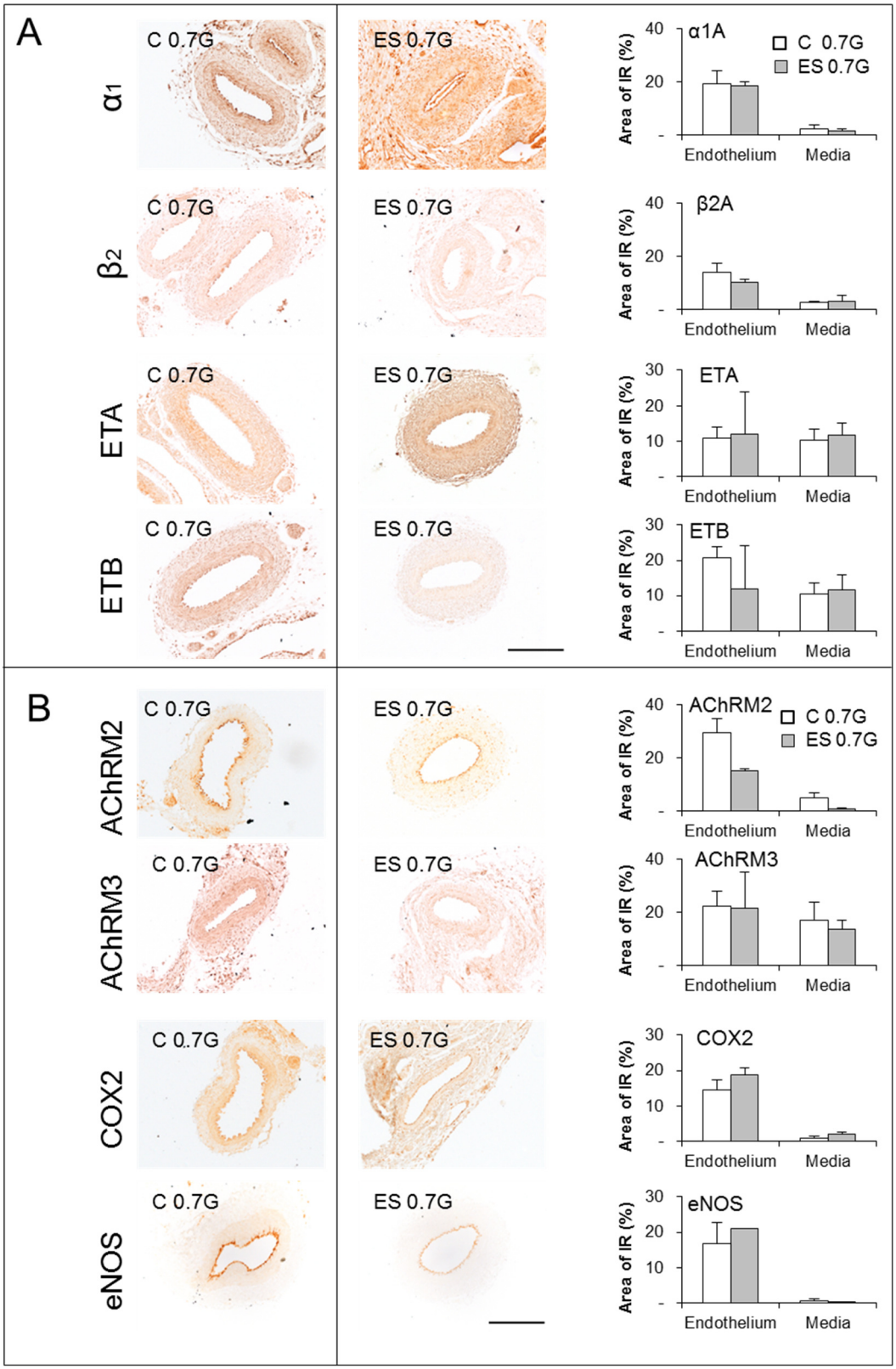


Fig. 7 Vascular effects of early stress on renal arteries at 0.7 gestation. A. effects on vasoconstrictive acting receptors B. effects on vasodilative acting receptors and enzymes. C 0.7 G: controls at 0.7 gestation. ES 0.7 G: early stress at 0.7 gestation. Area of IR (%) Area of imunoreactivity

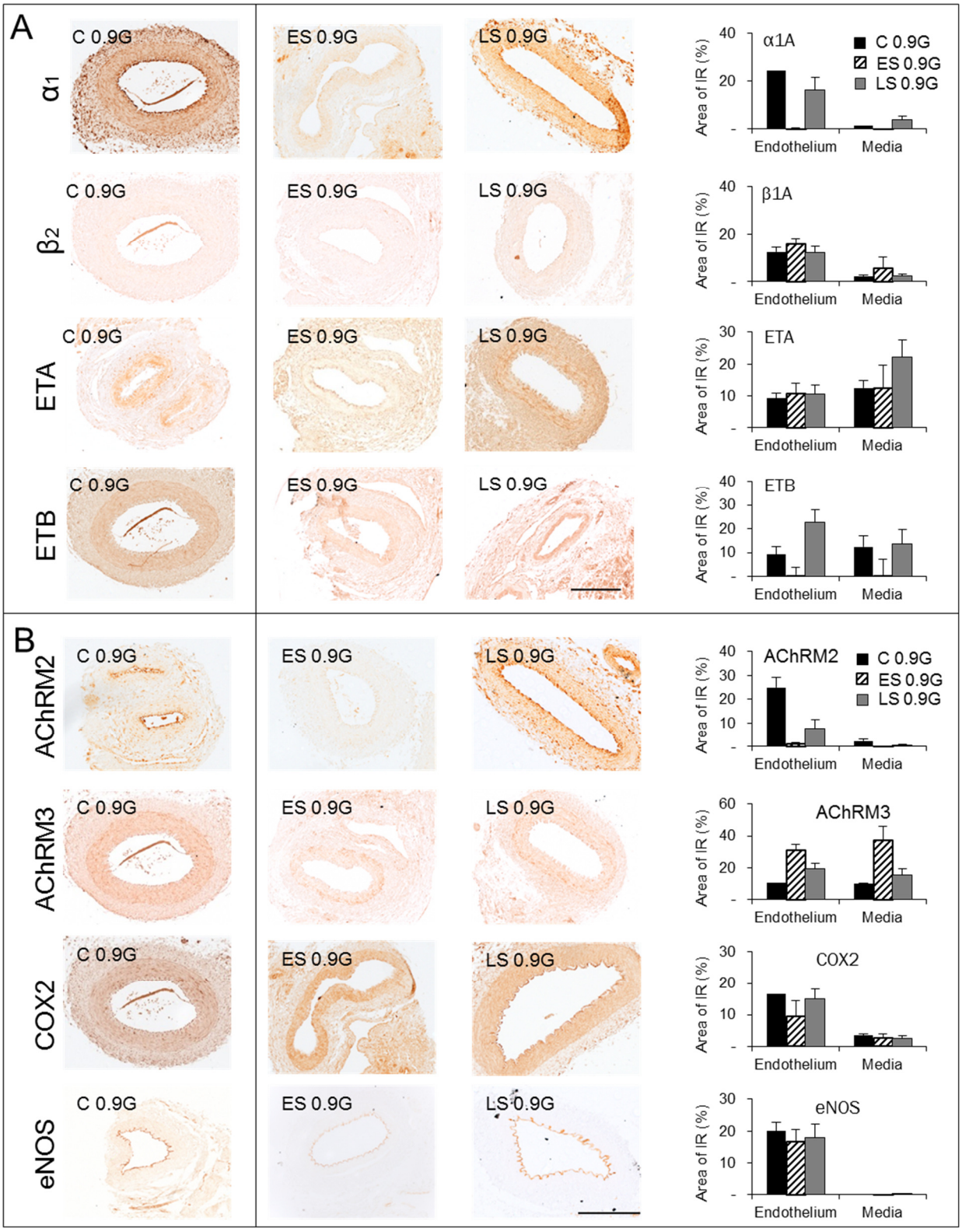


Fig. 8 Vascular effects of early stress on renal arteries at 0.9 gestation. A. effects on vasoconstrictive acting receptors B. effects on vasodilative acting receptors and enzymes. C 0.9 G: controls at 0.7 gestation. ES 0.9 G: early stress at 0.7 gestation. LS 0.9 G Late stress at 0.9 gestation. Area of IR (%) Area of imunoreactivity

Tables

Table 1

Table 1

	0.7 gestation	early stress 0.7 gestation	p	0.9 gestation	early stress 0.9 gestation	p	late stress 0.9 gestation	p
mesenteric arteries								
resting tension (mN)	1.66±0.15	1.53±0.37	0.77	3.21±0.65	1.56±0.38	0.04	2.12±0.19	0.17
normalized internal diameter (µm)	310.00±15.41	292.14±30.30	0.63	515.17±98.77	321.33±56.51	0.22	337.00±69.79	0.23
max. tension (mN)	4.64±0.52	3.40±0.71	0.20	7.18±1.69	6.21±0.84	0.86	10.03±1.07	0.25
vessel length (mm)	1.93±0.02	1.81±0.06	0.12	1.86±0.04	1.93±0.04	0.36	1.93±0.02	0.30
renal arteries								
resting tension (mN)	2.5±0.4	1.8±0.3	0.18	2.3±0.4	2.2±0.4	0.99	2.6±0.5	0.89
normalized internal diameter (µm)	369.7±22.9	362.6±26.6	0.84	445.5±52.0	404.3±55.6	0.85	406.4±51.6	0.87
max. tension (mN)	8.0±1.1	6.8±1.5	0.53	9.5±1.4	10.1±1.2	0.93	6.8±1.2	0.33
vessel length (mm)	1.92±0.03	1.88±0.03	0.46	1.75±0.08	1.88±0.03	0.26	1.87±0.06	0.34

Table 1. Descriptive parameter of fetal mesenteric and renal arteries. Data are mean ± SEM.

Table 2

	0.7 gestation			early stress 0.7 gestation		
	Endothelium	Media	Endothelium/ Media (%)	Endothelium	Media	Endothelium/ Media (%)
AChRM2	0.20±0.03*	0.02±0.01	24.4±7.3	0.15±0.04*	0.01±0.003	24.7±8.1
AChRM3	0.26±0.03*	0.17±0.01	1.6±0.1	0.17±0.05	0.14±0.04	1.3±0.3
α_{1A}	0.31±0,05*	0.05±0.02	9.5±2.6	0.28±0.05*	0.05±0.01	23.1±17.0
α_{2A}	0.09±0.04	0.03±0.01	2.9±0.6	-	-	-
β_2	0.36±0.11*	0.08±0.02	5.4±0.8	0.05±0.01*	0.01±0.01	6.01±1.4
COX-2	0.20±0.07*	0.06±0.03	9.2±2.7	0.14±0.04*	0.01.0.002	40.7±15.9
eNOS	0.20±0.07*	0.002±0.002	9022.9±3685.2	0.22±0.04*	0.002±0.001	557.7±248.8
ET_A	0.07±0.02	0.06±0.01	1.2±0.3	0.06±0.03	0.09±0.04	0.9±0.5
ET_B	0.21±0.06*	0.09±0.03	3.8±1.5	0.13±0.03*	0.06±0.01	3.0±0.8
EP2	0.11±0.04	0.08±0.04	3.0±1.5	0.11±0.03	0.11±0.04	1.5±0.5

	0.9 gestation			early stress 0.9 gestation			late stress 0.9 gestation		
	Endothelium	Media	Endothelium/ Media (%)	Endothelium	Media	Endothelium/ Media (%)	Endothelium	Media	Endothelium/ Media (%)
AChRM2	0.28±0.07*	0.03±0.01	36.3±14.4	0.07±0.02*	0.002±0.001	40.2±10.2	0.19±0.05*	0.01±0.002	30.9±7.2
AChRM3	0.18±0.06*	0.08±0.02	2.3±0.5	0.24±0.04	0.16±0.04	1.7±0.4	0.15±0.01*	0.1±0.03	2.0±0.3
α_{1A}	0.32±0.04*	0.02±0.01	219.2±202.1	0.15±0.04*	0.01±0.01	25.0±8.1	0.14±0.05*	0.04±0.02	84.8±71.6
α_{2A}	0.20±0.06	0.11±0.03	3.8±1.9	-	-	-	-	-	-
β_2	0.21±0.10	0.05±0.05	14.5±7.9	0.07±0.02*	0.03±0.01	5.4±2.5	0.09±0.03*	0.01±0.002	7.3±2.0
COX-2	0.25±0.05*	0.05±0.02	14.86.3	0.12±0.04*	0.01±0.01	42.6±22.6	0.08±0.03*	0.01±0.003	42.6±22.4

eNOS	0.15±0.06*	0001±0.0007	485.3±277.0	0.08±0.03	0.036±0.035	371.0±243.7	0.13±0.04*	0.0003±0.0001	766.3±215.3
ET_A	0.05±0.02	0.04±0.01	1.3±0.4	0.10±0.02	0.21±0.07	1.9±1.5	0.09±0.04*	0.18±0.04	0.6±0.2
ET_B	0.15±0.05*	0.11±0.05	4.4±2.3	0.06±0.03*	0.01±0.01	22.9±12.3	0.19±0.04*	0.08±0.02	3.3±0.8
EP2	0.07±0.02	0.02±0.01	5.6±2.3	0.10±0.05	0.15±0.05	8.8±8.2	0.10±0.03	0.11±0.03	1.0±0.2

Table 2: Fetal mesenteric artery receptor and enzyme expression using the ratio of area for immunoreactivity in endothelium and media following early stress at 0.7 and early and late stress at 0.9 of ovine gestation. Acetylcholine receptor M2 (AChRM2), muscarinic acetylcholine receptor M3 (AChRM3), adrenoreceptor α1A (α1A), adrenoreceptor α2A (α2A), adrenoreceptor β2 (β2), cycloogenase-2 (COX-2), endothelial NO synthase (eNOS), endothelin-1 receptor type A (ETA), endothelin-1 receptor type B (ETB), prostaglandin-E2 receptor EP2 (EP2), prostaglandin-E2 receptor EP4 (EP4). Data are mean ± SEM. *$p < 0.05$ expression in endothelium compared to media by paired students T-Test. # $p< 0.05$ Endothelium/media expression ratio of controls compared to stressed animals 0.7 and 0.9 gestation by oneway ANOVA (here: p always >0.05)

Table 3

	0.7 gestation			early stress 0.7 gestation		
	Endothelium	Media	Endothelium/ Media (%)	Endothelium	Media	Endothelium/ Media (%)
AChRM2	0.29±0.05*	0.05±0.02	9.26±2.59	0.15±0.04*	0.009±0.004	54.58±30.60
AChRM3	0.22±0.06	0.17±0.07	2.76±1.28	0.22±0.05	0.14±0.03	1.89±0.53
α_{1A}	0.19±0.05*	0.025±0.014	97.13±45.43	0.18±0.05*	0.06±0.007	28.61±9.19
β_2	0.14±0.03*	0.026±0.016	7.78±3.63	0.10±0.01*	0.034±0.022	17.18±11.98
COX-2	0.14±0.03*	0.01±0.005	36.13±16.04	0.14±.0.3*	0.01±0.001	44.74±24.16
eNOS	0.17±0.06*	0.006±0.005	691.26±331.32	0.21±0.04*	0.001±0.001	5479.57±2552.75
ET_A	0.11±0.03	0.1±0.03	1.23±0.26	0.12±0.03	0.12±0.03	1.41±0.36
ET_B	0.21±0.04*	0.11±0.04	4.43±1.95	0.30±0.06*	0.13±0.04	5.60±2.07

	0.9 gestation			early stress 0.9 gestation			late stress 0.9 gestation		
	Endothelium	Media	Endothelium/ Media (%)	Endothelium	Media	Endothelium/ Media (%)	Endothelium	Media	Endothelium/ Media (%)
AChRM2	0.25±0.04*	0.023±0.009	26.00±10.95	0.01±0.005	0.0003±0.0002	100.93±64.6	0.08±0.04	0.006±0.004	36.25±15.65
AChRM3	0.10±0.02	0.10±0.03	1.27±0.24	0.31±0.03	0.37±0.08	0.86±0.11	0.19±0.04	0.15±0.04	1.51±0.40
α_{1A}	0.22±0.04*	0.013±0.004	28.61±12.42	0.003±0.002	0.0001±0.00009	47.23±21.73	0.17±0.05*	0.039±0.016	76.71±52.55
β_2	0.10±0.02*	0.019±0.003	5.27±0.71	0.16±0.03	0.057±0.047	7.17±5.41	0.12±0.03*	0.023±0.008	6.36±1.08
COX-2	0.17±0.02*	0.04±0.01	5.83±1.35	0.09±0.05	0.03±0.02	3.95±0.46	0.15±0.03*	0.03±0.01	15.79±8.64
eNOS	0.20±0.03*	0.0005±0.0002	739.94±266.41	0.17±0.04	0.00007±0.00002	2813.02±1370.85	0.18±0.04*	0.0028±0.0012	384.54±306.67
ET_A	0.09±0.02	0.12±0.02	0.80±0.12	0.11±0.03	0.13±0.07	1.03±0.44	0.10±0.03*	0.22±0.05	0.49±0.08
ET_B	0.12±0.03	0.08±0.05	9.02±5.01	0.01±0.01	0.002±0.0003	4.45±4.22	0.23±0.05*	0.14±0.06	2.32±0.5

Table 3: Fetal renal artery receptor and enzyme expression using the ratio of area for immunoreactivity in endothelium and media following early stress at 0.7 and early and late stress at 0.9 of ovine gestation. Acetylcholine receptor M2 (AChRM2), muscarinic acetylcholine receptor M3 (AChRM3), adrenoreceptor α1A (α1A), adrenoreceptor β2 (β2), cycloogenase-2 (COX-2), endothelial NO synthase (eNOS), endothelin-1 receptor type A (ETA), endothelin-1 receptor type B (ETB). Data are mean ± SEM. *$p < 0.05$ expression in endothelium compared to media by paired students T-Test. # $p< 0.05$ Endothelium/media expression ratio of controls compared to stressed animals 0.7 and 0.9 gestation by oneway ANOVA (here: p always >0.05)

4.3 Manuscript 3: Cardiovascular effects of prenatal stress – Are there implications for cerebrovascular, cognitive and mental health outcome?

Neuroscience and Biobehavioral Reviews xxx (2018) xxx-xxx

Contents lists available at ScienceDirect

Neuroscience and Biobehavioral Reviews

journal homepage: www.elsevier.com

Review article

Cardiovascular effects of prenatal stress—Are there implications for cerebrovascular, cognitive and mental health outcome?

Julia J. Müller*, [1], Iwa Antonow-Schlorke[1], Nasim Kroegel, Sven Rupprecht, Florian Rakers, Otto W. Witte, Matthias Schwab

Hans Berger Department of Neurology, Jena University Hospital, Jena, Germany

ARTICLE INFO

Keywords:
Anxiety
Depression
Hypothalamus-pituitary-adrenocortical axis
Autonomic nervous system
Sympathetic activity
Maternal-fetal circulation
Uterine and umbilical blood flow
Cerebral blood flow
Vascular reactivity
Blood pressure

ABSTRACT

Prenatal stress programs offspring cognitive and mental health outcome. We reviewed whether prenatal stress also programs cardiovascular dysfunction which potentially modulates cerebrovascular, cognitive and mental health disorders. We focused on maternal stress and prenatal glucocorticoid (GC) exposure which have different programming effects. While maternal stress induced cortisol is mostly inactivated by the placenta, synthetic GCs freely cross the placenta and have different receptor-binding characteristics. Maternal stress, particularly anxiety, but not GC exposure, has adverse effects on maternal-fetal circulation throughout pregnancy, probably by co-activation of the maternal sympathetic nervous system, and by raising fetal catecholamines. Both effects may impair neurodevelopment. Experimental data also suggest that severe maternal stress and GC exposure during early and mid-gestation may increase the risk for cardiovascular disorders. Human data are scarce and especially lacking for older age. Programming mechanisms include aberrations in cardiac and kidney development, and functional changes in the renin-angiotensin-aldosterone-system, stress axis and peripheral and coronary vasculature. Adequate experimental or human studies examining the consequences for cerebrovascular, cognitive and mental disorders are unavailable.

Nomenclature

Index of abbreviations

ANGII	Angiotensin-II
AT_1	Angiotensin-II receptor
BM	Betamethasone
DOHaD	Developmental Origins of Health and Disease
DM	Dexamethasone
eNOS	Endothelial nitric oxide synthase
ET-1	Endothelin-1
ET_A	Endothelin-1 type A receptor
ET_B	Endothelin-1 type B receptor
GC	Glucocorticoid
GR	Glucocorticoid receptor
HPA axis	Hypothalamus-pituitary-adrenocortical axis
L-NAME	L-arginine methyl ester hydrochloride
LBW	Low birth weight
MCA	Middle cerebral artery
MR	Mineralocorticoid receptor
NO	Nitric oxide
PI	Pulsatility index
PIV	Pulsatility index for veins
RAAS	Renin-angiotensin-aldosterone-system
RI	Resistance index
SAS	Sympathetic-adrenomedullary system
S/D ratio	Systolic/diastolic ratio
SNP	Sodium nitroprusside (NO-donor)
TXA_2	Thromboxane-A2
TXA_2-R	Thromboxane-A2 receptor
U-46619	Thromboxane-A2 mimetic
UA	Umbilical artery
UtA	Uterine artery
VSMCs	Vascular smooth muscle cells
WHO	World Health Organization

* Corresponding author at: Hans Berger Department of Neurology, Jena University Hospital, Am Klinikum 1, 07747 Jena, Germany.
Email address: julia.mueller@med.uni-jena.de (J.J. Müller)

[1] Authors contributed equally to the paper.

https://doi.org/10.1016/j.neubiorev.2018.05.024
Received 20 April 2017; Received in revised form 21 May 2018; Accepted 22 May 2018
Available online xxx
0149-7634/

11β-HSD2	11β-hydroxysteroid dehydrogenase type 2

1. Introduction

Prenatal exposure to adverse environmental influences during critical periods of development determines offspring health in later life to a great extent ('Developmental Origins of Health and Disease (DOHaD) hypothesis'). Prenatal stress and low nutrient availability comprise major adverse prenatal environmental influences affecting the development of multiple fetal organ systems such as the cardiovascular system and the brain as well as their function in later life. Prenatal stress, i.e. increased fetal exposure to stress hormones or to indirect effects of maternal stress (Rakers et al., 2017a,b), mainly results from maternal stress and therapeutic glucocorticoid (GC) administration which shall be the focus of this review (Fig. 1). Other sources of prenatal stress such as malnutrition, smoking, substance abuse, preeclampsia and obesity are beyond the scope of this review since they may have direct (i.e. stress hormone independent) effects on fetal development. According to the studies available, we consider the following types of maternal stress: (1) maternal psychological distress including nonspecific perceived psychological stress, pregnancy-specific stress, anxiety and depression, and (2) negative life events experienced by the pregnant woman such as illnesses in the close family or financial and relationship problems About of pregnant women in industrialized countries and of pregnant women in low and middle income countries report maternal stress during pregnancy (UNFPA, 2008). Studying the effects of maternal stress is complicated by the fact that stress perception is subjective. In general, designing an experimental stress model relevant to the human situation has proved challenging. To overcome the subjective view and study the effects of maternal stress objectively, several researchers have examined the therapeutic administration of synthetic GCs on fetal development and offspring outcome (Anwar et al., 2016; Fowden and Forhead, 2015; Millage et al., 2016; Moisiadis and Matthews, 2014b). Synthetic GCs such as betamethasone (BM) and dexamethasone (DM) are widely used to accelerate fetal lung maturation in babies at risk of preterm delivery (Panel, 2001). Annually,

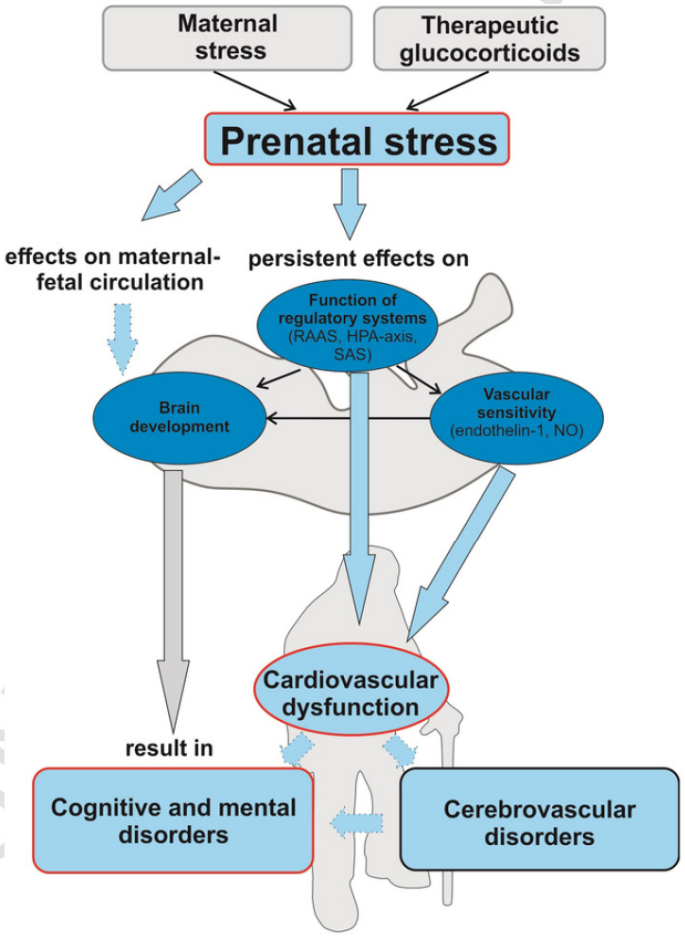


Fig. 1. Potential relationships between prenatal stress, cardiovascular dysfunction and cognitive and mental disorders in later life. The direct effects of prenatal stress fetal malnutrition maternal stress and therapeutic glucocorticoids the direct of prenatal stress on cognitive and mental health disorders are not considered in this review (grey arrows). Dotted arrows show relationships that have not yet been sufficiently proven.

around 15 million or of pregnant women worldwide deliver preterm (Blencowe et al., 2012) and GC therapy is administered to % of these mothers (Boesveld et al., 2014; Chandrasekaran and Srinivas, 2014; Lee et al., 2011). Synthetic GCs are used therapeutically since they easily cross the placenta and are not a substrate of placental 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2). The enzyme 11β-HSD2 inactivates of maternal cortisol (corticosterone in rats) and, thus, partially protects the fetus from maternal stress (Harris and Seckl, 2011). It is important to note that the effects of synthetic GCs on the fetus may differ from the effects of maternal stress in several ways. Maternal stress can be transferred from mother to fetus not only by cortisol but also by other mechanisms such as the catecholamine-mediated impairment of uterine blood supply (Rakers et al., 2017a,b). The latter does not occur after synthetic GC treatment (Schwab et al., 2006). Endogenous GCs such as cortisol or corticosterone in rodents bind to both glucocorticoid receptors (GR) and mineralocorticoid receptors (MR) but synthetic glucocorticoids bind predominantly to GR (Kliewer et al., 1998). GR are ubiquitously expressed in the brain while MR are mainly located in the limbic system (Ahima et al., 1991). Activation of GR produce neurotoxic and apoptosis-inducing effects (Hassan et al., 1996; Packan and Sapolsky, 1990) and MR induce neuroprotective effects (Hassan et al., 1996). Saturation of MR occurs at much lower steroid concentrations than saturation of GR because MR have approximately a tenfold higher affinity for cortisol / corticosterone (Reul and Kloet, 1985). Cortisol at higher concentrations, typical for stressed individuals (McEwen et al., 1987), and synthetic GCs activate GR and induce neurotoxic effects (Hassan et al., 1996). Moreover, the biological potency of synthetic GCs is higher comparedto that of cortisol (Yang et al., 1990a). In the vasculature, GR and MR receptors are expressed in both endothelial and vascular smooth muscle cells (VSMCs) but activate different pathways (Tarjus et al., 2015; Yang and Zhang, 2004).

The fetal cardiovascular system and brain are particularly vulnerable to prenatal stress due to their long period of development and intrinsic plasticity (Bock et al., 2015; Brunton, 2015; Dyer and Rosenfeld, 2011; Polanska et al., 2017; Souza et al., 2017; Thornburg, 2015). This may occur as a result of the direct effect on the developmental trajectory of the brain and the vascular system or by changes in the activities of functional systems such as the renin-angiotensin-aldosterone-system (RAAS) and the stress axis with its two limbs, the hypothalamus-pituitary-adrenocortical (HPA) axis and the sympathetic-adrenomedullary system (SAS) which affect brain and cardiovascular function in later life (for reviews see (Anwar et al., 2016; Kapoor et al., 2006).

The effects of maternal stress and therapeutic prenatal GC exposure on offspring cognitive, behavioral and mental health problems in later life as well as the underlying changes in neurodevelopment and activity of the stress axis are well studied (van den Bergh, this issue). Less studied are the effects of maternal stress and prenatal GC exposure on the function of the cardiovascular system during both fetal and postnatal life (McMillen and Robinson, 2005; Nijland et al., 2008; Palinski, 2014). These effects are also of major interest with regard to the cognitive and mental health outcomes for several reasons. Firstly, disturbances in maternal-fetal circulation and fetal cerebrovascular function may affect neurodevelopment by restriction of nutrient and oxygen availability (Fig. 1). The developing brain consumes about 50% of the nutrient and oxygen supply (Gibbons, 1998). A severe decrease in nutrient supply, for example via disturbances in maternal-fetal circulation can lead to restriction in fetal growth in general (Malhotra et al., 2017a). Despite a compensatory redistribution of blood flow to the brain, fetal brain development is frequently altered under such conditions (Miller et al., 2016; Wang et al., 2016). Secondly, prenatal stress may program offspring cardiovascular dysfunction either by changing the function of the stress axis and the RAAS, or by programming vascular sensitivity to vasomediators (Fig. 1). Programming of vascular sensitivity may result in cardiovascular dysfunction and cerebrovascular disorders which could both be linked to cognitive and mental health disorders (Fig. 1). It has been suggested that a decrease in brain perfusion is due to alterations in the cerebral vasculature, rather than due to changes in the neural activity that underlies age-related cognitive changes during brain aging (Bell et al., 2010; Li and Freeman, 2010; Martin et al., 1991). Alteration in the contractility of cerebral vessels may diminish the supply of oxygen, energy substrates and nutrients to the brain. The importance of an adequate fuel supply for brain function is highlighted by the fact that although the brain comprises ~2% of total body mass, it receives as much as 20% of cardiac output and is responsible for ~20% and ~25% of the body's oxygen and glucose consumption, respectively (Nelson et al., 2016). Mild hypoperfusion affects protein synthesis which is required for synaptic plasticity, and for mediating learning and memory. In fact, moderate to severe reductions in cerebral blood flow (CBF) and hypoxia can affect ATP synthesis and the ability of neurons to generate action potentials (Kalaria, 2010).

Here, we review the acute, short-term (<7 days), long-term (<30 days), and persistent effects of maternal stress and prenatal GC exposure on maternal-fetal circulation as well as fetal cardiovascular and cerebrovascular development and function in later life (Fig. 1). We elucidate the potential mechanisms by which maternal stress and prenatal GC exposure affect maternal-fetal circulation and program the cardiovascular system taking into account the translational value of experimental research for the human situation. We search for knowledge on the relationship between fetal programming of cardiovascular function by maternal stress and prenatal GC exposure and cerebrovascular, cognitive and mental health outcomes. A comprehensive knowledge of the relevant mechanisms involved is invaluable to identify innovative targets for early prevention of cardiovascular as well as cerebrovascular, cognitive and mental health diseases.

2. Effects of prenatal stress on maternal-fetal circulation and fetal cardiovascular development

2.1. Human studies

2.1.1. Effects of maternal stress

Since impairment of placental circulation is considered a key mechanism by which maternal stress may affect the human fetus (Gitau et al., 2001; Rakers et al., 2017a, b), several studies investigated a possible relationship between different types of maternal stress (Table 1) and maternal-fetal circulation. A total of 13 prospective studies met the following criteria: use of standardized methods for assessing maternal stress, use of ultrasound Doppler sonography to measure fetal and placental hemodynamics, and provision of an adequate description of the study methods. Of these, eight studies were longitudinal: two studies over the entire pregnancy (Roos et al., 2015; Vythilingum et al., 2010), three studies during the second and third trimesters (Harville et al., 2008; Maina et al., 2008; Mendelson et al., 2011), one study over the second trimester (Monk et al., 2012), and two studies during the third trimester (Helbig et al., 2014; Sjostrom et al., 1997). Five of the 13 studies were prospective studies in which ultrasound Doppler sonography was conducted at single time-points during the second (Çalışkan et al., 2009; Helbig et al., 2011; Kent et al., 2002) or third trimester (Helbig et al., 2013; Teixeira et al., 1999). Several Doppler ultrasound indices of the uterine artery (UtA) and umbilical artery (UA) describing vascular resistance (resistance index (RI), pulsatility index (PI) and systolic/diastolic (S/D) ratio) were measured. Blood flow volume of the umbilical vein and other parameters of fetal circulation such as fetal ductus venosus PI for veins (PIV), middle cerebral artery (MCA) PI, and the cerebroplacental ratio (MCA PI / UA PI) were also determined.

2.1.1.1. Maternal-fetal circulation Anxiety and depression. A pilot study that included 30 pregnant women assessed at gestational weeks showed a slight positive correlation between self-reported trait anxiety

Table 1
Psychometric tests used in human studies for assessment of mental well-being and maternal stress.

psychometric measures	psychometric scale	study
mental health		
emotional well-being	WHO Well-being Index	Mendelson et al. 2011
uplifts specific to pregnancy	Pregnancy Experiences Scale	Mendelson et al. 2011
active coping	John Henryism Scale	Harville et al. 2008
maternal stress		
psychological distress	28-item version General Health Questionnaire	Helbig et al. 2011A, Helbig et al. 2011B, Helbig et al. 2013, Helbig et al. 2014
	Paykel scoring of stressful life events	Maina et al. 2008
	Kessler-10 scale	Vythilingum et al. 2010, Roos et al. 2015
	Sarason's Life Experience Survey	Harville et al 2008
	Cohen Perceived Stress Scale,	Çalışkan et al. 2009, Harville et al. 2008, Vythilingum et al. 2010, Roos et al. 2015
	22-item Impact of Event Scale, incl. Subscales: intrusion: intrusive and unbidden thoughts avoidance: emotional numbness arousal: psychophysiological symptoms	Helbig et al. 2011B, Helbig et al. 2013, Helbig et al. 2014
anxiety	Spielberger State-Trait Anxiety Inventory	Sjostrom et al. 1997, Harville et al. 2008, Vythilingum et al. 2010, Mendelson et al. 2011, Roos et al., 2015
	Hospital Anxiety and Depression Score	Kent et al. 2002
	Hamilton Rating Scale for Anxiety	Maina et al. 2008, Monk et al. 2012
depressive mood	Beck Depression Inventory	Monk et al. 2012
	Edinburgh Postnatal Depression Scale	Helbig et al. 2014
	Hospital Anxiety and Depression Score	Kent et al. 2002
	Hamilton Rating Scale for Depression	Maina et al. 2008
	Center for Epidemiologic Survey Depression Scale	Mendelson et al. 2011
neuropsychiatric disorders	Mini International Neuropsychiatric Interview Plus	Maina et al. 2008
	Structured Clinical Interview for Diagnosis	Monk et al. 2012

and uterine resistance (UtA PI) which, however, did not persist after adjusting for alcohol and nicotine use (Vythilingum et al., 2010). A more extended study by the same group that included 75 healthy pregnant women showed that higher scores for trait anxiety but not for state anxiety were correlated with an increased UtA PI (Roos et al., 2015). In another study, Vythilingum et al. found a weak positive correlation between trait anxiety and uterine resistance (UtA PI) in 79 pregnant women assessed at gestational weeks (Vythilingum et al., 2010) and which were comparable to results obtained at gestational weeks. Using a similar study protocol and a larger population of 140 healthy pregnant women, Roos et al. replicated the correlation between higher trait anxiety scores and increased UtA PI (Roos et al., 2015). Positive correlations for self-reported state and trait anxiety scores during the third trimester with uterine resistance (UtA RI) were also found in another study including 100 healthy pregnant women (Teixeira et al., 1999). In addition, mothers with high state anxiety showed more notches in the waveform pattern than low state anxiety mothers also indicative for abnormal maternal-fetal circulation (Teixeira et al., 1999). The study could not distinguish between short-term and long-term effects of anxiety since the top 15% of women with either state or trait anxiety were largely the same patients. However, long-term effects of trait anxiety were supported by a study in 59 women who showed a weak positive correlation between trait anxiety reported during the first or second-trimester and uterine resistance (UtA PI) in the third trimester (Vythilingum et al., 2010). In contrast, no striking associations between anxiety or depression during the course of pregnancy and uterine resistance during the second or third trimester were found in a large study in 872 women (Harville et al., 2008) and in two further studies, each including 107 pregnant women (Helbig et al., 2013; Mendelson et al., 2011). Moreover, no acute, short term or long term relationship between measures of anxiety and depression during the first and second trimester and uterine resistance during the second trimester (Monk et al., 2012) were seen in a study by Monk et al in 101 women, all with lifetime histories of psychiatric disorders. Similarly, Kent et al. did not find an association between definite cases of anxiety and uterine resistance (UtA RI) during the second trimester (Kent et al., 2002).

Focusing on umbilical circulation, a study in 37 pregnant women showed increased umbilical resistance (UA PI) in women with self-rated higher trait but not state anxiety scores (Sjostrom et al., 1997). The UA RI and UA S/D ratio but not the UA PI was found increased in 60 pregnant women suffering high anxiety during the period between indication and performance of amniocentesis (Çalışkan et al., 2009). But the lack of change in the UA PI represents the more sensitive parameter for disturbances in maternal-fetal circulation (Gomez et al., 2008; Roos et al., 2015). However, most studies failed to show a relationship between anxiety measures and umbilical resistance, independent of gestational age (Harville et al., 2008; Helbig et al., 2013; Mendelson et al., 2011; Roos et al., 2015; Vythilingum et al., 2010).

Maternal psychological distress. No associations between maternal stress and uterine or umbilical resistance were found in most of the studies independent of the stage of pregnancy (Helbig et al., 2013, 2014; Mendelson et al., 2011; Roos et al., 2015; Vythilingum et al., 2010). Even the occurrence of a distinct and major negative incidence specific to pregnancy such as diagnosis of fetal malformation did not induce an association between distress level and uterine and umbilical artery resistance one to five days after diagnosis in a study of 86 pregnant women (Helbig et al., 2011). However, increased uterine resistance (UtA PI) during the third trimester was seen in women who experienced psychological distress due to a diagnosis of an axis I psychiatric disorder (Vythilingum et al., 2010). Contrary to the hypothesis of an maternal stress -mediated increase in resistance in maternal-fetal circulation, a longitudinal analysis between the second and third trimester by Mendelson et al. (2011) surprisingly revealed that a higher distress level is modestly associated with lower UtA RI in the right uterine artery and psychological well-being with lower UtA RI in the left uterine artery (Mendelson et al., 2011). These findings point to differential effects of maternal stress on the left and right uterine arteries which could have been overlooked in studies that did not differentiate between the uterine arteries. Apart from uterine and umbilical resistance, two studies also measured umbilical vein blood flow volume. Intrusion scores correlated negatively with the umbilical vein volume blood flow normalized for fetal growth, and therefore, support a decrease in feto-

placental blood flow as a possible pathway between maternal stress and reduced fetal growth (Helbig et al., 2013). Another study by the same group showed that psychological distress (intrusion level) and depressive symptoms during the second but not third trimester antedated increased umbilical vein blood flow volume during the third trimester in 74 pregnant women with a history of a structural malformation in a previous fetus or offspring (Helbig et al., 2014). While the former study supports a decrease in fetoplacental blood flow as a possible pathway between maternal stress and reduced fetal growth, the latter study does not.

Thus, three out of eight studies indicate a mainly trait anxiety-associated increase in uterine resistance independent of the stage of pregnancy (Roos et al., 2015; Teixeira et al., 1999; Vythilingum et al., 2010). The remaining five, mostly larger studies did not show such an association (Harville et al., 2008; Helbig et al., 2013; Kent et al., 2002; Mendelson et al., 2011; Monk et al., 2012). Regarding the umbilical circulation, only two (Çalışkan et al., 2009; Sjostrom et al., 1997) out of six studies (Harville et al., 2008; Helbig et al., 2013; Mendelson et al., 2011; Monk et al., 2012; Roos et al., 2015; Vythilingum et al., 2010) showed a relationship between maternal anxiety and umbilical resistance. At the very least, these findings point to disturbances in maternal-fetal circulation, particularly in high anxiety mothers. Differences in study design, participant numbers and profiles, measurement of flow parameters, and presence of cofounders may explain the inconsistent study results. No relationship was found between acute, high and non-specific psychological distress and increased uterine or umbilical resistance in healthy pregnant women (Helbig et al., 2011, 2013, 2014; Mendelson et al., 2011; Roos et al., 2015; Vythilingum et al., 2010). Measuring the uterine and umbilical resistance is probably a crude method for detecting disturbances in the maternal-fetal circulation. Measuring umbilical vein blood flow, however, appears to be more sensitive since high intrusion levels were associated with a decrease in umbilical vein blood flow that may be associated to lower fetoplacental blood flow (Helbig et al., 2013). Moreover, increase in the resistance of the maternal-fetal circulation might be compensated by an increase in placental size (Helbig et al., 2014). This increase in placental size could explain why the majority of longitudinal studies do not support placental blood flow reduction as a pathway between maternal distress and reduced fetal growth. Notably, even when the studies were longitudinal, the study design did not allow for a differentiation between chronic or repeated maternal psychological distress. Consequently, there is no clear evidence firstly that maternal stress is associated with a decreased maternal-fetal circulation and secondly that it may be involved in the link between maternal stress and brain development.

2.1.1.2. Fetal systemic and cerebral circulation Fetal systemic and cerebral perfusion was assessed with regard to the effects of maternal stress during the second and third trimester by examining the ductus venosus PIV (Roos et al., 2015) and MCA PI (Roos et al., 2015; Sjostrom et al., 1997; Teixeira et al., 1999; Vythilingum et al., 2010). There was no evident relationship between measures of maternal psychological distress or anxiety and fetal ductus venosus PIV (Roos et al., 2015). In contrast, negative correlations were seen between third-trimester trait anxiety scores and fetal vascular resistance of the MCA (MCA PI) as well as the cerebro-umbilical ratio (Sjostrom et al., 1997). Similarly, a high maternal anxiety state was a short-term predictor of lower vascular resistance of the MCA (MCA PI) during the third but not the second trimester (Roos et al., 2015). Although these studies indicate changes in fetal circulation in favor of cerebral perfusion, other findings did not indicate a relationship between psychological distress or anxiety and MCA PI (Roos et al., 2015; Teixeira et al., 1999; Vythilingum et al., 2010). Hence, the relationship between maternal stress and cerebral perfusion in humans has not yet been clearly proven.

2.1.2. Effects of prenatal glucocorticoid exposure

2.1.2.1. Uterine and umbilical circulation In pregnancies complicated by the absence of end diastolic flow in the UAs, treatment with BM to enhance fetal lung maturation in threatened premature labor induced an acute transient return of end diastolic blood flow in a high percentage of pregnancies (Barkehall-Thomas et al., 2003; Edwards et al., 2002, 2003; Muller et al., 2003; Robertson et al., 2009; Wallace and Baker, 1999) without changes in umbilical resistance (Muller et al., 2003). In contrast, Kähler et al found a transient decrease in umbilical resistance (UA RI) 30 min after maternal BM administration that was reversible within 24 h (Kähler et al., 2004). The decrease in UA RI may reflect a decrease of placental vascular resistance and/or increased venous return (Schwab et al., 2006). The return of the end diastolic blood flow might be due to a prolonged increase in UA blood flow owing to an increase in fetal heart rate, and thus, cardiac output (Schwab et al. 2006). In contrast to studies in fetal sheep (Schwab et al. 2006), human studies failed to show any effect of BM on maternal-fetal circulation (Chitrit et al., 2000; Cohlen et al., 1996; Deren et al., 2001; Piazze et al., 2001; Rotmensch et al., 1999; Senat and Ville, 2000).

2.1.2.2. Fetal systemic and cerebral circulation In systemic circulation, the fetal branch pulmonary arteries showed an acute decrease in resistance indices in response to BM administration in threatened preterm labor (Bartha et al., 2008). The vasodilatory effect of BM in fetal branch pulmonary arteries is similar to the effect seen in fetal sheep (Crossley et al., 2009) and indicates increased pulmonary perfusion in line with the lung maturational effect of GCs. In contrast, an acute transient increase in peak systolic velocity of the ductus arteriosus was reported after maternal BM injection that returned to baseline within 8 h (Kähler et al., 2004). BM and DM had no effect on the vascular resistance of the ductus venosus (Cohlen et al., 1996; Kähler et al., 2004; Muller et al., 2003), hepatic vein and inferior vena cava (Kähler et al., 2004), fetal renal artery (Cohlen et al., 1996) and the descending aorta (Cohlen et al., 1996; Senat and Ville, 2000).

Studies examining the effects of GCs on fetal cerebral circulation showed heterogeneous results. For example, maternal BM or DM administration reduced vascular resistance of the MCA (MCA PI) within 24 or within 48 h in fetuses with both normal and disturbed fetoplacental blood flow, indicating an improvement in fetal cerebral perfusion (Chitrit et al., 2000; Edwards et al., 2002; Muller et al., 2003). Here, reduction in MCA resistance by synthetic GCs seems to be most effective in fetuses younger than 32 gestational weeks (Piazze et al., 2001). Although BM and DM effects were reversible within days (Chitrit et al., 2000; Piazze et al., 2001), Urban et al. found a decreased vascular resistance of the MCA (MCA PI) and cerebro-umbilical ratio 5 days after the first dose of DM (Urban et al., 2005). In contrast, a study by Cohlen et al. and by other groups showed no short-term BM-induced changes in the resistance of the middle (Cohlen et al., 1996; Deren et al., 2001; Kähler et al., 2004; Rotmensch et al., 1999), anterior and posterior cerebral arteries (Cohlen et al., 1996). These findings indicate acute and short-term decreases of cerebral resistance following treatment with synthetic GCs, but these results have not been reported with consistency. There are no studies examining the long-term effects of fetal GC exposure on fetal systemic and cerebral circulation.

2.2. Animal studies

Animal models allow a more systematic approach for studying the direct effects of both maternal stress and prenatal GC exposure on fetal cardiovascular development and their underlying mechanisms, in contrast to epidemiologic studies. Apart from rodents, important animal models for such studies include ruminants and non-human primates. The latter two species are closer to humans than rodents. Moreover,

their larger size allows the study of both fetal cardiovascular physiology after chronic instrumentation and vascular reactivity *ex-vivo* using small vessel myography. Various species-specific stressors have been used to study maternal psychosocial distress in a number of animal models. For example, rats and guinea pigs were exposed to restraint stress (Igosheva et al., 2004, 2007), noise and light (Kapoor and Matthews, 2005; Weinstock et al., 1998) as well as social instability (Kaiser et al., 2015) whereas in sheep, shearing (Corner et al., 2010) and social isolation (Roussel et al., 2004) were used as maternal psychological stressors. In non-human primates, bright light was utilized to induce maternal psychosocial distress (Morishima et al., 1978). Additional data on the effects of prenatal stress on fetal cardiovascular function is derived from experimental studies directly investigating the effects of therapeutic GC exposure on fetal circulation. Despite a number of advantages provided by animal models, they are also associated with a few disadvantages. For instance, the effects of social and behavioral stress models may differ from those due to maternal stress in humans. Moreover, development of cardiovascular diseases in later life is difficult to simulate in animal models. Therefore, outcome parameters describing the cardiovascular function such as heart rate and blood pressure were used in studies to estimate adverse intrauterine effects on the programming of fetal and offspring cardiovascular constitution.

2.2.1. Effects of maternal stress

There are very few data from animal studies describing the effects of maternal psychosocial distress on fetal cardiovascular function (Morishima et al., 1978; Rakers et al., 2015). In sheep, maternal psychological distress in the form of maternal social isolation resulted in an acute but transient catecholamine-mediated decrease in uterine blood flow accompanied by an increase in fetal cortisol and norepinephrine concentrations and fetal blood pressure (Rakers et al., 2015) (Fig. 2). In non-human primates, fetal blood pressure was also raised transiently during maternal agitation induced by bright light (Morishima et al., 1978) (Fig. 3). However, effects of maternal stress on umbilical or fetal cerebral circulation have not yet been investigated.

2.2.2. Effects of prenatal glucocorticoid exposure

In contrast to humans, considerably more research data is available on the effects of GCs, in particular on the effect of administration of maternal DM or BM on fetal cardiovascular function (Fig. 2).

Acute effects. Maternal DM or BM in sheep during late gestation (0.7–0.85 of gestation) increased fetal blood pressure within hours of

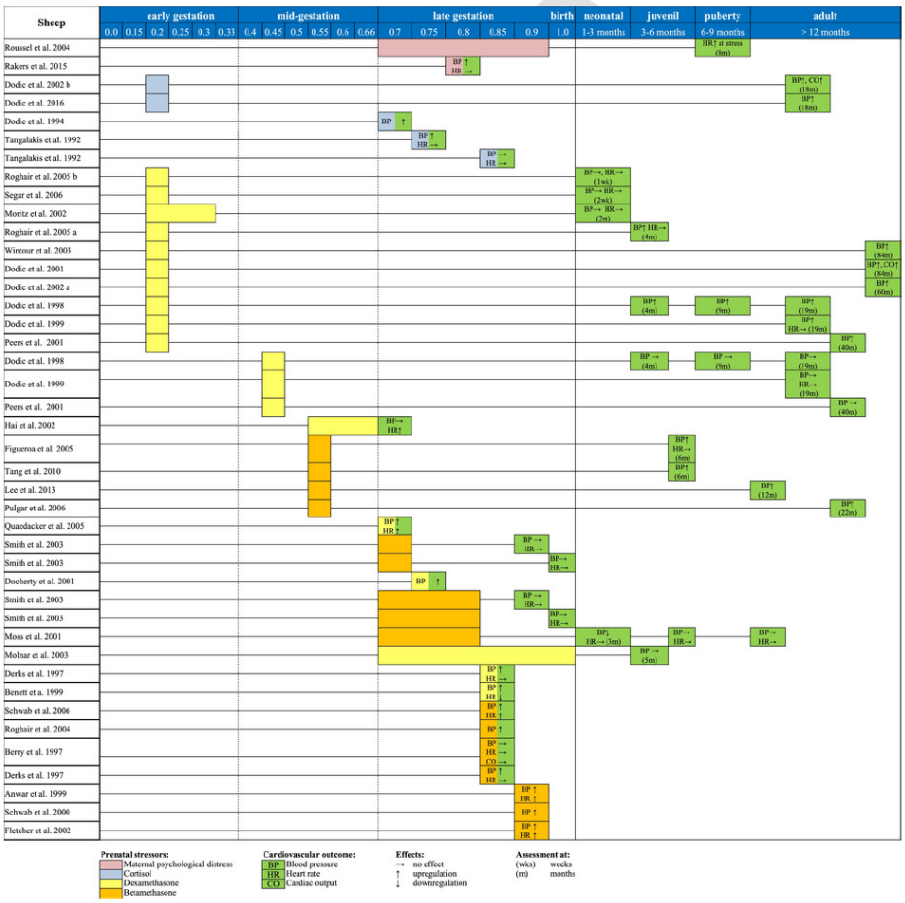


Fig. 2. Cardiovascular outcome after maternal psychosocial distress and prenatal glucocorticoid exposure in sheep. Note the persistent blood pressure increase after prenatal glucocorticoid exposure during early and mid-gestation but not during late gestation.

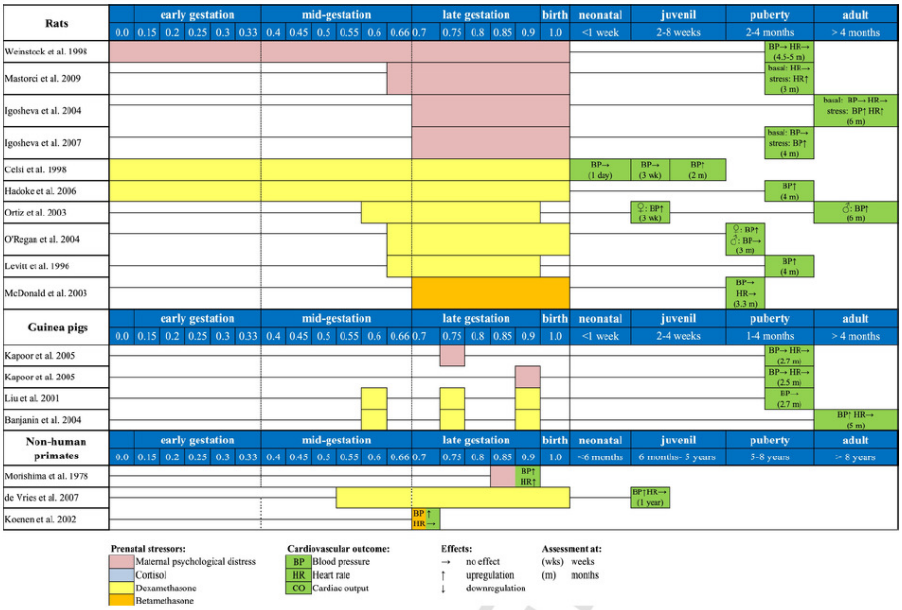


Fig. 3. Cardiovascular outcome after maternal psychosocial distress and prenatal glucocorticoid exposure in rats, guinea pigs and non-human primates. Note the increased blood pressure in adult rat offspring after prenatal glucocorticoid exposure during late gestation. Since the rat is an altricial species, late gestation is comparable to earlier gestational ages in precocial species. Maternal psychosocial stress during late gestation does not program baseline blood pressure but prolongs stress-induced blood pressure increase in the rat offspring.

administration (Bennet et al., 1999; Berry et al., 1997; Quaedackers et al., 2005; Schwab et al., 2006), which is comparable to findings in pregnant baboons at 0.7 of gestation (Koenen et al., 2002). Similarly, direct fetal administration of DM or BM in late gestation sheep (0.75–0.85 of gestation) induced an immediate increase in fetal blood pressure (Anwar et al., 1999; Berry et al., 1997; Derks et al., 1997; Docherty et al., 2001; Fletcher et al., 2002; Roghair et al., 2004; Schwab et al., 2000). These increases in blood pressure were transient and normalized within 48 h. In contrast, direct cortisol infusion to the sheep fetus resulted in an acute increase of fetal blood pressure when administered at 0.7, but not at 0.86 of gestation (Dodic and Wintour, 1994; Tangalakis et al., 1992). Older fetuses may be protected from inappropriate high cortisol concentrations by the enzyme 11β-HSD2 since its expression increases in fetal tissues with advancing gestation (Brown et al., 1996; Speirs et al., 2004).

GC-induced increase in fetal blood pressure in sheep was accompanied by changes in blood flow. Maternal administration of BM led to a transient increase of umbilical blood flow which closely correlated to an increase in fetal heart rate suggesting that the transient increase of umbilical blood flow is due to an increase in cardiac output (Schwab et al., 2006). In contrast, maternal GC treatment decreased cerebral and femoral blood flow (Fletcher et al., 2002; Lohle et al., 2005; Miller et al., 2012; Quaedackers et al., 2005; Schwab et al., 2000). This decrease in cerebral and femoral blood flow was mediated by an increase in vascular resistance (Lohle et al., 2005; Miller et al., 2012; Schwab et al., 2000). Such an increase in vascular resistance could also be observed in the carotid and femoral arteries (Derks et al., 1997; Quaedackers et al., 2005).

Long-term effects. In agreement with the transient acute increase in fetal blood pressure following maternal or fetal DM or BM administration in late gestation sheep (Anwar et al., 1999; Berry et al., 1997; Derks et al., 1997; Docherty et al., 2001; Fletcher et al., 2002; Roghair et al., 2004; Schwab et al., 2000), there was no increase in fetal blood pressure one week after BM administration (0.9 of gestation) (Smith et al., 2003). In contrast, DM administration during early gestation (0.2 of gestation) led to a long-term increase in fetal blood pressure (0.85 of gestation) (Roghair et al., 2005a) which, however, did not seem to persist into the neonatal period (Moritz et al., 2002; Roghair et al., 2005b; Segar et al., 2006).

Taken together, GC effects on fetal circulation in sheep have almost exclusively been examined during late gestation. GC exposure of the fetus induces an acute but transient increase of blood pressure which is mediated by an increase in fetal cardiac output and vascular resistance. The increase in vascular resistance also involves the cerebral circulation and results in a decreased fetal cerebral blood flow.

2.3. Summary

Although the available data on the effects of maternal stress on maternal-fetal circulation in human studies is inconsistent, it nevertheless suggests that changes are caused by anxiety rather than by maternal psychological distress and are independent of the stage of pregnancy. The inconsistency in the results may be due to the differences in the stress type and stress intensity examined in the studies. High anxiety seems to preferentially increase uterine resistance (Roos et al., 2015; Teixeira et al., 1999; Vythilingum et al., 2010) which is in agreement with studies in sheep showing a maternal stress -associated and catecholamine-mediated decrease in uterine blood flow (Dreiling et al., 2016; Rakers et al., 2015). The maternal stress -associated increase in uterine resistance may, consequently, jeopardize fetal nutrient and oxygen supply. Although this decrease in uterine blood flow only occurs at the beginning of stress periods in sheep, it induces a prolonged shift toward an anaerobic metabolic state in the fetus (Dreiling et al., 2016, 2017; Rakers et al., 2015). This effect, however, seems to be mediated by the fetal catecholamine release associated to the decrease in uterine blood flow rather than directly by a decrease in placental nutri-

ent supply. It further appears that the adverse effects of maternal stress on uterine-placental circulation may trigger a compensatory lasting increase in placental size (Helbig et al., 2014) which could compensate for the chronic maternal stress -induced fetal (catecholamine-triggered) metabolic shift toward an anaerobic metabolism. Indeed, sheep fetuses were better oxygenated after chronic stress without a shift towards acidosis, potentially reflecting adaptation to chronic stress (Dreiling et al., 2017). However, there is little evidence from human studies on long-term effects of maternal stress on maternal-fetal circulation.

While human studies on the effects of maternal stress and GCs on peripheral vascular resistance are largely lacking, experimental studies have examined the effects of GCs on fetal circulation in sheep mostly during late gestation (Fig. 2). Exposure of the fetus to maternal stress (Dreiling et al., 2017; Rakers et al., 2015) and GCs (Derks et al., 1997; Quaedackers et al., 2005; Schwab et al., 2006; Tangalakis et al., 1992) induces an acute but transient increase in blood pressure which is mediated by an increase in fetal cardiac output and vascular resistance. GCs administered to pregnant sheep during early gestation induce a lasting increase in blood pressure in the fetus which, however, (Roghair et al., 2005a) does not persist into the neonatal period (Moritz et al., 2002; Roghair et al., 2005b; Segar et al., 2006). The increase in vascular resistance due to maternal GC administration also involves the cerebral circulation and results in decreased fetal cerebral blood flow (Lohle et al., 2005; Schwab et al., 2000). In contrast, maternal stress (Roos et al., 2015; Sjostrom et al., 1997) and administration of synthetic GCs (Chitrit et al., 2000; Edwards et al., 2002; Muller et al., 2003; Piazze et al., 2001) resulted in a decreased resistance in the cerebral vasculature in human studies, although these results could not be reproduced in other studies (Cohlen et al., 1996; Deren et al., 2001; Kähler et al., 2004; Rotmensch et al., 1999; Teixeira et al., 1999; Vythilingum et al., 2010). The decrease in vascular resistance of brain feeding arteries in parallel to the increase in resistance in uterine-placental circulation suggests improved cerebral perfusion which may reflect a brain-sparing effect (Malhotra et al., 2017b). However, these results need to be confirmed in future studies since they are in contrast to studies in fetal sheep where synthetic GC administration transiently increases the resistance of the cerebrovascular system and, thereby, decreases cerebral perfusion (Lohle et al., 2005; Miller et al., 2009; Quaedackers et al., 2005; Schwab et al., 2000).

3. Effects of prenatal stress on cardiovascular function in the offspring

3.1. Human studies

3.1.1. Effects of maternal stress

There are very few studies in the literature linking maternal stress to cardiovascular disease in later life compared to the numerous studies examining the relationship between low birth weight (LBW) and cardiovascular disease (for reviews see (Järvelin et al., 2004; Raaijmakers et al., 2017; Ylihärsilä et al., 2004). In the Amsterdam Born Children and their Development study, depressive symptoms, state anxiety, pregnancy-related anxiety, daily parenting stress, and job strain were recorded by pregnant women during the early 2 trimester using questionnaires (van Dijk et al., 2012). The presence of psychosocial stressors in pregnant women was associated with a 1.5 mmHg higher systolic and diastolic blood pressure in the offspring at years of age (van Dijk et al., 2012). However, at the age of years, offspring showed lower diastolic blood pressure (van Dijk et al., 2014), raising the question whether the slight increase in blood pressure at years of age in this cohort is actually related to maternal stress. A recently published study from Denmark provides potential evidence for an association between maternal stress and cardiovascular dysfunction in the offspring (Plana-Ripoll et al., 2016). This large population-based study included 2,607,851 participants born between to mothers who suffered the loss of a relative either during pregnancy or a short time before pregnancy. In this study, subjects who were followed for up to 40 years of age, showed a modest association between prenatal stress and cardiovascular dysfunction or arterial hypertension.

3.1.2. Effects of prenatal glucocorticoid exposure

There is some evidence, albeit inconsistent, showing that antenatal therapy using synthetic GCs results in higher blood pressure in human neonates. Whereas two studies report a rise in blood pressure extremely premature infants (Moise et al., 1995) and at 14 years of age (Doyle et al., 2000), others found no blood pressure increase in adults at 20 or 30 years of age compared to placebo (Dalziel et al., 2005; Dessens et al., 2000). In detail, the study by Moise et al. in 240 extremely premature infants born between 23 and 27 weeks gestation demonstrated that infants whose mothers received prenatal GCs showed higher blood pressure during the first 48 h of life (Moise et al., 1995). Similarly, in a long-term follow-up study of 177 adolescents whose mothers received one course of antenatal BM therapy, and who were born preterm with a birth weight < 1501 g, subjects showed a higher systolic and diastolic blood pressure at the age of 14 years compared to adolescents whose mothers received no prenatal treatment (Doyle et al., 2000). In contrast, a randomized controlled trial showed that 81offspring who were born preterm or term from mothers treated with BM to enhance fetal lung maturation developed lower systolic blood pressure without differences in diastolic blood pressure at the age of 20 years (Dessens et al., 2000). Similarly, a large scale follow-up of a randomized controlled trial in 534 young adults whose mothers received BM or placebo during pregnancy could not demonstrate a difference in blood pressure or a history of cardiovascular disorders at the age of 30 years (Dalziel et al., 2005).

Other studies compared single versus repeated courses of BM. A prospective cohort study in 50 infants born prematurely at 32 weeks gestation and exposed to multiple courses of synthetic GCs had higher blood pressure in the first week of life compared to single course exposure (Mildenhall et al., 2006). Moreover, the end diastolic interventricular septal wall thickness was found increased by >2 SD above the age-adjusted normal value in these infants (Mildenhall et al., 2006). On the other hand, no effects of multiple vs. single courses of BM on blood pressure and myocardial wall thickness were seen by the same research group in 145 newborns at 6 weeks in a randomized controlled trial (Mildenhall et al., 2009). In a follow-up of 258 children (123 treated with BM) between the ages of years, repeated antenatal BM treatment of mothers did not increase potential cardiovascular risk factors including 24-hour ambulatory blood pressure compared to a single antenatal BM course (McKinlay et al., 2015). Taken together, there is no clear evidence that antenatal BM treatment to enhance fetal lung maturation affects long-term cardiovascular outcome even when BM treatment was repeated.

3.2. Animal studies

3.2.1. Effects of maternal stress

Persistent effects of maternal psychological distress on cardiovascular function in the offspring have mainly been investigated in rats, guinea pigs and sheep (Igosheva et al., 2004; Igosheva et al., 2007; Kapoor and Matthews, 2005; Mastorci et al., 2009; Roussel et al., 2004; Weinstock et al., 1998) (Fig. 3). All the above studies consistently showed no alteration in baseline blood pressure by maternal psychological distress in adult offspring (Igosheva et al., 2004; Igosheva et al., 2007; Kapoor and Matthews, 2005; Mastorci et al., 2009; Weinstock et al., 1998). Similarly, offspring of guinea pigs that were exposed to stroboscopic light during late pregnancy (0.73 or 0.86 of gestation) did not show elevated basal blood pressure or plasma cortisol levels (Kapoor

and Matthews, 2005). In contrast, an augmented systolic blood pressure response to acute stress during adulthood was observed in prenatally stressed rats (Igosheva et al., 2004; Igosheva et al., 2007; Mastorci et al., 2009). Likewise, neonatal sheep born to ewes stressed by maternal isolation during late pregnancy showed increased heart rate during the startling stimulus stress test (Roussel et al., 2004). Thus, although maternal psychological distress did not affect basal cardiovascular parameters in the offspring, exposure to stressful challenges showed enhanced stress-sensitivity and impaired blood pressure regulation in prenatally stressed offspring.

3.2.2. Effects of prenatal glucocorticoid exposure

Maternal DM administration during pregnancy increased basal blood pressure in adult offspring of rats and guinea pigs (Banjanin et al., 2004; Celsi et al., 1998; Hadoke et al., 2006; Levitt et al., 1996; Liu et al., 2001; O'Regan et al., 2004) (see Fig. 3). In contrast, BM or hydrocortisone administered to rats in late pregnancy did not elevate blood pressure in adult offspring despite similar experimental protocols (Celsi et al., 1998; McDonald et al., 2003; Moritz et al., 2005). Here, the lack of change in blood pressure may be explained by the fact that the natural corticosteroid, hydrocortisone, is inactivated to a large extent by placental 11β-HSD2. However, the reason why BM, which is not a substrate for 11β-HSD2, did not induce elevated blood pressure in offspring in the study by McDonald et al. remains unclear (McDonald et al., 2003).

Although DM administered in early ovine pregnancy (0.2 of gestation) induced increased fetal blood pressure during late gestation (Roghair et al., 2005a), this blood pressure increase did not persist into the early postnatal period (Moritz et al., 2005; Roghair et al., 2005b; Segar et al., 2006) (Fig. 2). Interestingly, the increased blood pressure became apparent again at 4 months of age (Dodic et al., 1998) and lasted until adulthood (5 years of age) (Dodic et al., 2001, 2002a, 1998, 2006, 1999, 2002b; Peers et al., 2001; Wintour et al., 2003). Similarly, administration of BM to pregnant sheep at mid-gestation (0.55 of gestation) also programmed increased blood pressure in juvenile (6 months of age) and adult offspring months of age) (Figueroa et al., 2005; Lee et al., 2013; Pulgar and Figueroa, 2006; Tang et al., 2010) Thus, the rise in blood pressure seems to become more pronounced with increasing age (Figueroa et al., 2005; Peers et al., 2001; Roghair et al., 2005b). While increased blood pressure after DM treatment during early gestation (0.2 of gestation) in sheep was associated with increased cardiac output including left ventricular hypertrophy but with no changes in peripheral resistance (Dodic et al., 2001, 1999), BM administration at mid-gestation (0.55 of gestation) induced increased peripheral vascular resistance in the adult offspring (Lee et al., 2013). However, cardiac output was not measured in the latter study. In contrast to GC administration during early and mid-gestation, BM administration during late gestation did not lead to elevated blood pressure in the adult sheep offspring (Moss et al., 2001). Taken together, fetal exposure to GCs affects offspring baseline blood pressure and the cardiovascular response to stress as seen after maternal psychological distress.

3.3. Summary

The very limited number of human studies, which includes one large cohort study, showed an inconsistent weak association between maternal stress and increased blood pressure in child- and early adulthood. Similarly, there is no clear evidence that antenatal BM treatment to enhance fetal lung maturation affects long-term cardiovascular outcome even after repeated BM treatment. However, most of the studies include small numbers of subjects and use varying study designs. For example, they did not differentiate between preterm and term born offspring and did not analyze any potential gender-related differences. Moreover, it is possible that baseline blood pressure used as a main outcome parameter is not a sensitive enough indicator to detect minor cardiovascular dysfunction. More in-depth phenotyping of cardiovascular function should be obtained in future studies. Similarly, maternal stress did not affect basal cardiovascular parameters such as blood pressure in the rat, guinea pig and sheep offspring but impaired blood pressure regulation during stress challenges. In contrast to maternal stress, fetal exposure to synthetic GCs in experimental studies seems to have stronger programming effects on cardiovascular function since it affects offspring baseline blood pressure. In sheep, GC-induced programming of increased blood pressure becomes apparent in the juvenile offspring. The more pronounced effects of synthetic GCs may be due to selective inactivation of maternal cortisol and corticosterone by placental 11β-HSD2 (Seckl and Meaney, 2004) and the higher biological potency of synthetic GCs (Yang et al., 1990b). More and larger prospective studies in humans following prenatal GC treatment are needed - albeit difficult to perform – that also examine cardiovascular responses to stress challenges.

.

The cardiovascular effects in the offspring resulting from prenatal stress may be due to an increase in peripheral resistance or cardiac output. Increased vascular tone can either be the consequence of alterations in vascular composition or functional activity. If not stated otherwise, all results discussed below were obtained in sheep studies after prenatal GC administration.

4.1. Cardiac effects of prenatal stress

The acute GC-induced increase in fetal blood pressure seems to be mediated by both a rise in cardiac output (Tangalakis et al., 1992; Schwab et al., 2006) and peripheral vascular resistance (Derks et al., 1997; Quaedackers et al., 2005). In sheep, programming of increased blood pressure in later life resulting from prenatal GC exposure during early gestation is associated with a left ventricular hypertrophy and increased cardiac output (Dodic et al., 2001, 1999). Similarly, a higher systolic blood pressure and an increase in heart rate in response to acute stress in adult rats after maternal stress exposure also suggests programming of cardiac output (Igosheva et al., 2004, 2007; Mastorci et al., 2009). Nevertheless, increased peripheral resistance also seems to play a prominent role in mediating the programming effects on blood pressure (Lee et al., 2013).

4.2. Vascular effects of prenatal stress

4.2.1. Alterations in vascular composition

There are some hints that the programming effects of prenatal GC exposure on blood pressure involve changes in structural vascular development (McMillen and Robinson, 2005). Matuszek et al. provide evidence for increased α-actin content in fetal sheep aortas after prenatal GC exposure (Matuszek et al., 2006). Similarly, LBW was linked to increased aortic stiffness in a cohort of 50-year olds (Martyn et al., 1995). Increased aortic stiffness is associated with a decrease in aortic elastin content (Nuyt, 2008). Hence, Martyn et al. suggested that reduced intrauterine growth impairs synthesis of elastin in the walls of the aorta and large arteries which may lead to permanent changes in the mechanical properties of these vessels predisposing to higher blood pressure, increased left-ventricular mass, and cardiovascular disease (Martyn and Greenwald, 1997). Whether vascular stiffness is limited to large conduit vessels has not been elucidated yet. Investigating the effect of prenatal stress on structural maturation of vasculature could be an important approach for future research to determine the mechanisms by which prenatal stress programs cardiovascular dysfunction.

4.2.2. Alterations in vascular function

Although GCs do not cause direct GR-mediated functional effects on the vascular tone, they are known to regulate vascular reactivity by suppression of vasodilator signaling and enhancing the effects of vasoconstrictor agents on endothelial cells and VSMCs (Yang and Zhang, 2004). Most of the studies on vascular reactivity have been performed in sheep using small vessel myography. In the fetus, GCs increase vascular resistance (Derks et al., 1997; Fletcher et al., 2002). The increase in peripheral vascular resistance is driven by an altered vasoconstrictor response to potassium chloride (KCl), adrenergic receptor agonists, endothelin-1 (ET-1), angiotensin-II (ANGII), thromboxane-A2 (TXA_2) mimetic U-46619 as well as an impaired vasodilator response to acetylcholine and nitric oxide (NO) (Table. 2) (Anwar et al., 1999; Docherty et al., 2001; Dodic et al., 1998; Eckman et al., 2010; Hai et al., 2002; Lee et al., 2013, 2014; Molnar et al., 2003, 2002; Pulgar and Figueroa, 2006; Roghair et al., 2004, 2005a,b; Tangalakis et al., 1992).

Potassium chloride. KCl, acting as an unspecific depolarizing vascular agent, it mediates an endothelium and receptor-independent vasoconstriction via a Ca^{2+} influx mainly involving L-Type Ca^{2+} channels (Blood et al., 2002; KARAKI et al., 1984). Thus, the vasoconstrictor response to KCl offers general information on the VSMC vasoconstrictor capacity. The magnitude of the vasoconstrictor response depends on the VSMC density as well as on the different aspects of VSMC function including myosin chain phenotype, L-type Ca^{2+} channel density and intracellular Ca^{2+}-homeostasis (Blood et al., 2002; Chern et al., 1995; Hai et al., 2002). The vasoconstrictor response to KCl depends on vascular bed and maturation of blood vessels. KCl-mediated vascular contractility increases with gestational age and reflects increasing medial wall thickness and maturation of the VSMC function (Bevan and Bevan, 1981; Hutanu et al., 2007).

GC exposure increases the fetal vasoconstrictor response to KCl acutely in femoral but not in mesenteric, coronary and middle cerebral arteries (Anwar et al., 1999; Docherty et al., 2001; Roghair et al., 2004). Potential mechanisms for the increased KCl-induced vasoconstriction include increased L-type channel density and possibly an accelerated maturation of the sarcoplasmic reticulum (Blood et al., 2002; Hai et al., 2002). However, these mechanisms are currently not well addressed in the literature. The acute effects of fetal GC exposure on KCl-mediated vascular contractility did not persist into adolescence or adulthood (Eckman et al., 2010; Molnar et al., 2003; Roghair et al., 2005a,b; Segar et al., 2006).

Endothelin-1. ET-1 is a major endothelium-derived vasomediator with mainly vasoconstrictor but also vasodilator properties. The vascular response depends on the expression of different ET-1 receptors in the VSMCs and the endothelium. The vasoconstrictor response to ET-1 is mediated by VSMC ET_A and ET_B receptors whereas the endothelial ET_B receptor mediates vasorelaxation primarily by the release of prostacyclin and nitric oxide (Haynes and Webb, 1994; Okawa et al., 2004). ET-1 is involved in the fetal vascular regulation at 0.7 of ovine gestation (Docherty et al., 2001). GC administration from mid- to late gestation results in an increased vasoconstrictor response to ET-1 in major conduit arteries such as the brachial and coronary arteries (Lee et al., 2014; Roghair et al., 2005b) and in small femoral resistance arteries (Docherty et al., 2001; Molnar et al., 2003, 2002). The increase in vascular tone in response to ET-1 in small resistance arteries such as femoral and mesenteric arteries (Docherty et al., 2001; Molnar et al., 2002) was dependent on the gestational age at GC exposure. GC administration during mid- and late but not early gestation resulted in an increased vascular resistance (Docherty et al., 2001; Molnar et al., 2003, 2002; Segar et al., 2006). Fetal middle cerebral arteries however, display a tachyphylactic response to ET-1 after GC exposure (Docherty et al., 2001). The GC-induced increased vasoconstrictor response to ET-1 seems to persist into adolescence in contrast to the transiently increased KCl-response (Molnar et al., 2003). The GC-induced increase of the ET-1 vasoconstrictor response is initiated by a change in receptor function, i.e. increased ET_A receptor binding or a diminished ET_B receptor activity and not by changes in receptor expression (Docherty et al., 2001; Lee et al., 2013; Molnar et al., 2003). Docherty et al. speculate that in middle cerebral arteries, tachyphylaxis is due to a GC-induced reduction of ET_A receptor binding although this phenomenon requires further investigation (Docherty et al., 2001).

Adrenergic receptor agonists. The effects of catecholamines on vascular function are mediated by the vasoconstrictor action of α1-receptors and the vasodilator action of β_2 adrenergic receptors on VSMCs. The most potent agonist for α_1- receptors is phenylephrine followed by norepinephrine and isoprenaline. The order of potency for β_2 adrenergic receptor agonists follows a reverse order: isoprenaline, norepinephrine, and phenylephrine. Maturation of the adrenergic-mediated vascular response parallels the maturation of the sympathetic branch of the autonomic nervous system and, thus, sympathetic vascular innervation (Nichols, 1993).

Fetal exposure to GCs does not appear to influence fetal adrenergic-mediated vascular regulation (Tangalakis et al., 1992). Systemic prenatal GC administration during late gestation did not have a long-term effect on the fetal blood pressure increase in response to fetal systemic norepinephrine infusion (Tangalakis et al., 1992) or on the *ex vivo* adrenergic-mediated vasoconstriction in different vascular beds (femoral and mesenteric resistance arteries, carotid and coronary arteries) at late gestation (Anwar et al., 1999; Hai et al., 2002; Roghair et al., 2004). Consequently, no effects of prenatal GC administration on adrenergic-mediated vasoconstriction were observed in juvenile offspring (Dodic et al., 1998; Molnar et al., 2003; Roghair et al., 2005a; Segar et al., 2006). Therefore it is unlikely that the vascular response to catecholamines is related to the stress-mediated programming of increased blood pressure in later life.

Angiotensin-II. ANGII plays a key role in the renin-angiotensin-dependent regulation of blood pressure. The vasoconstrictor effect of ANGII is mediated by the ANGII receptor AT_1. AT_1 receptors appear early in gestation (0.1-0.2 of human gestation) with maximum expression in tissues regulating cardiovascular, fluid, and electrolyte homeostasis (Hu et al., 2004; Schütz et al., 1996).

In fetal sheep, direct systemic infusion of ANGII induced a profound increase in fetal blood pressure at 0.8 of gestation which was augmented by GCs (Tangalakis et al., 1992). Blood pressure increase was paralleled by an increased vasoconstrictor ANG II response and overexpression of AT_1 receptors in the coronary but not mesenteric arteries (Roghair et al., 2004). The absence of a GC-induced increase of vascular tone in the resistance vessels in the presence of increased fetal blood pressure after ANGII infusion suggests that GC exposure affects ANGII-mediated renal fluid and sodium retention. In postnatal life (at months of age), this GC effect was absent (Dodic et al., 1998). At the vascular level, GC exposure at 0.2 of gestation did not increase fetal or neonatal ANGII response in the carotid and mesenteric arteries (Roghair et al., 2005a,b) but small coronary arteries demonstrated a GC-dependent increase in vascular response to ANGII at 4 months of age (Roghair et al., 2005a). However, fetal blood pressure increase during ANGII infusion did not change after GC administration during early gestation (Dodic et al., 1998).

To conclude, GC-mediated enhancement of the ANGII-mediated blood pressure increase seems to be a fetal phenomenon whereby GCs affect renal fluid and sodium retention (Hutanu et al., 2007; Schütz et al., 1996) rather than peripheral vascular resistance. Moreover, programming of increased blood pressure in later life by prenatal GC exposure does not seem to be ANGII-mediated.

Thromboxane A_2 The potential effects of TXA_2 have been examined using the thromboxane A_2 mimetic U-46619. TXA_2 is one of the most

Table 2
Effects of prenatal glucocorticoid exposure on vasoreactivity of peripheral resistance, conduit and brain feeding vessels in sheep. (For interpretation of the references to color in this Table legend, the reader is referred to the web version of this article.)

	Resistance vessels	Conduit vessels	Brain feeding vessels	Vascular effect	References
KCl	mesenteric →	coronary →		l	Roghair et al. 2005 a
	mesenteric →	coronary →	carotid →	p	Roghair et al. 2005 a
		coronary →		p	Roghair et al. 2005 b
	femoral →			p	Segar et al. 2006
	mesenteric →			p	Segar et al. 2006
	femoral ↑			s	Anwar et al. 1999
	femoral →		MCA →	s	Docherty et al. 2001
	mesenteric ↓	coronary →		s	Roghair et al. 2004
	femoral ↑			l	Molnar et al. 2002
	femoral →			p	Molnar et al. 2003
			MCA →	p	Eckman et al. 2010
Endothelin-1		coronary ↑		l	Roghair et al. 2005 a
	femoral →			p	Segar et al. 2006
	mesenteric ↓			p	Segar et al. 2006
	femoral ↑		MCA tachyphylaxis	s	Docherty et al. 2001
	femoral ↑			l	Molnar et al. 2002
	femoral ↑			p	Molnar et al. 2003
		brachial ↑		p	Lee et al. 2014
Adrenergic receptor agonists:					
Phenylephrine	mesenteric →			p	Roghair et al. 2005 a
	mesenteric →			p	Roghair et al. 2005 b
Norepinephrine	femoral ↓			p	Segar et al. 2006
	mesenteric →			p	Segar et al. 2006
	femoral →			s	Anwar et al. 1999
			carotid →	s	Hai et al. 2002
	femoral →			p	Molnar et al. 2003
Isoprenaline	mesenteric →	coronary →		s	Roghair et al. 2004
Angiotensin-II	mesenteric ↓	coronary →		l	Roghair et al. 2005 a
	mesenteric →	coronary ↑	carotid →	p	Roghair et al. 2005 a
		coronary →		p	Roghair et al. 2005 b
	mesenteric →	coronary ↑		s	Roghair et al. 2004
Thromboxane-A2	mesenetric →	coronary →		l	Roghair et al. 2005 a
	mesenetric →	coronary ↑	carotid ↑	p	Roghair et al. 2005 a
		coronary →		p	Roghair et al. 2005 b
	femoral →			s	Anwar et al. 1999
	mesenteric →	coronary →		s	Roghair et al. 2004
Acetylcholine	mesenteric →	coronary ↑	carotid non-responsive	p	Roghair et al. 2005 a
	femoral ↓			s	Anwar et al. 1999
	femoral →		MCA non-responsive	s	Docherty et al. 2001
	mesenteric ↓	coronary ↑		s	Roghair et al. 2004
	femoral ↓			p	Molnar et al. 2003
Sodium nitroprusside		coronary ↑		p	Roghair et al. 2005 b
	mesenteric ↑	coronary →		s	Roghair et al. 2004
	femoral →			p	Molnar et al. 2003
		brachial →		p	Lee et al. 2014

Glucocorticoid treatment:		**Vascular effects:**		
Early gestation (blue)	→	no change in vascular resistance	s	acute and short-term
Mid- to late gestation (red)	↑	increased vascular resistance	l	long-term
	↓	decreased vascular resistance	p	persistent

important vasoconstrictors in fetal and placental circulation (Parisi and Walsh, 1989) and acts via the TXA_2 receptor TXA_2-R (Ogletree, 1987). GC exposure during early or late gestation had no effect on the fetal vasoconstrictor response to U-46619 in coronary, mesenteric and femoral arteries (Anwar et al., 1999; Roghair et al., 2004, 2005a). But GC administration at 0.2 of gestation enhanced U-46619-mediated vasoconstriction in coronary and carotid arteries at 4 months of age (Roghair et al., 2005a). No GC effects were observed in the mesenteric resistance arteries. The absent a GC effect on the U-46619-mediated vasoconstriction in resistance vessels suggests that TXA_2 neither mediates a GC-induced acute fetal blood pressure increase nor programs blood pressure regulation. Nevertheless, the increase of the vasoconstrictor response to U-46619 in coronary and carotid arteries in 4-month old offspring after GC exposure during early but not late gestation suggests a region-specific programming GC effect (Roghair et al., 2005a). Underlying mechanisms, such as TXA_2-R expression were not addressed and need to be clarified in future research.

Acetylcholine. Acetylcholine is the main neurotransmitter in the parasympathetic nervous system. Vasoregulator effects of acetylcholine are mediated by muscarinic acetylcholine receptors that are expressed in VSMC and endothelial cells. The muscarinic receptor M2, which is expressed on VSMC has vasoconstrictor properties whereby the "classical "vasodilator effects of acetylcholine are mediated by the endothelial M3 receptors via release of NO and prostaglandins (Eglen and Whiting, 1990).

The vasodilator response to acetylcholine increases with gestational age indicating functional maturation of the endothelium during mid- and late gestation (Furchgott and Zawadzki, 1980; Nishina et al., 2003). However, the vascular effects of acetylcholine differ between different vascular beds. Whereas acetylcholine elicits vasodilation in fetal resistance and brachial arteries, brain feeding arteries such as the carotid and middle cerebral artery are unresponsive to acetylcholine (Docherty et al., 2001; Roghair et al., 2005a) probably due to a lack of autonomic innervation. In contrast, coronary arteries show vasoconstrictor effects to acetylcholine (Anwar et al., 1999; Docherty et al., 2001; Roghair et al., 2004, 2005a). The paradoxical vasoconstriction induced by acetylcholine in coronary arteries is most likely due to the altered expression of endothelial M3 receptors and seems to be involved in endothelial dysfunction important for coronary vasospasm (Ludmer et al. 1986; Eglen and Whiting, 1990; Yasue et al., 1990).

Immediately following prenatal GC exposure during late gestation, dilator responses to acetylcholine in fetal femoral and mesenteric resistance arteries were enhanced (Anwar et al., 1999; Roghair et al., 2004). Additionally, fetal coronary arteries showed increased vasoconstriction (Roghair et al., 2004). These effects of fetal GC administration seem to persist into adulthood. Early, mid-, and late gestational GC exposure enhanced acetylcholine-dependent vasorelaxation in femoral and brachial arteries and increased vasoconstriction in coronary arteries of neonatal, juvenile and adult offspring (Molnar et al., 2003; Pulgar and Figueroa, 2006; Roghair et al., 2005a).

To sum up, since prenatal GC administration leads to an enhancement of both the vasoconstrictor acetylcholine response in coronary arteries and the vasodilator response in resistance arteries, acetylcholine most likely does not mediate programming of increased blood pressure in later life. It is more likely that the paradoxical vasoconstriction in coronary arteries reflects a deficient endothelial vasodilator function which could promote the pathogenesis of atherosclerosis in later life (Ludmer et al., 1986).

Nitric oxide induced relaxation. NO, the major vasodilator in the vasculature, is produced by endothelial nitric oxide synthase (eNOS) and induces endothelium- and receptor -independent vascular relaxation. The vascular function of NO was tested either with the NO donor sodium nitroprusside (SNP) (Lee et al., 2014; Molnar et al., 2003; Roghair et al., 2004, 2005a) or by inhibition of eNOS with nitro-L-arginine methyl ester hydrochloride (L-NAME) (Molnar et al., 2003). In femoral resistance arteries, a profound response to SNP was already present at 0.5 gestation (Nishina et al., 2003) reflecting early maturation of receptor-independent vasorelaxation.

GC exposure in late gestation has no acute effect on fetal coronary artery response to SNP and on the expression of eNOS (Roghair et al., 2004). Exposure to GCs during early gestation, results in a diminished SNP-induced coronary but not femoral vasorelaxation in neonatal offspring without affecting eNOS expression (Roghair et al., 2005b; Segar et al., 2006). In contrast, mid- gestational administration of GCs does not alter vascular SNP responses and eNOS expression in femoral or brachial arteries of juvenile and adult offspring (Lee et al., 2014; Molnar et al., 2003). Thus, early but not mid- gestational GC exposure seems to result in persistently reduced SNP-induced vasorelaxation in coronary arteries whereas eNOS expression is not affected. These effects may contribute to coronary artery dysfunction in older age.

In summary, prenatal GCs have a multitude of programming effects on vasomediators whereby the effects vary in different vascular beds. Peripheral resistance vessels and coronary arteries are more susceptible than brain feeding vessels which may reflect the brain-sparing effect with regard to the increase in vascular resistance after maternal psychological distress and prenatal GC exposure. GCs program an increase in vascular tone in peripheral resistance vessels and coronary arteries as well as vascular remodeling in big conduit vessels. Although these results were obtained in experimental studies, the changes may represent potential mechanisms accelerating the development of hypertension, coronary artery disease and atherosclerosis in humans.

4.3. Effects of prenatal stress on humoral regulation of cardiovascular function

4.3.1. Autonomic regulation and hypothalamus-pituitary-adrenocortical axis activity

Sympathetic-adrenomedullary system. During fetal life, maternal stress -induced increase in UtA and UA vascular resistance is most likely catecholamine-mediated (Rakers et al., 2015). Similarly, psychological or psychiatric disorders during pregnancy are associated with autonomic dysregulation in the fetus (Monk et al., 2000, 2003; Sjostrom et al., 2002). Fetal autonomic dysregulation may also impact cardiovascular development (Monk et al., 2000, 2003; Sjostrom et al., 2002) since catecholaminergic and serotonergic neurons constitute the essential components of central neural cardiovascular control (Dampney, 1994). In agreement with this, development of vagal tone was delayed in neonates from high anxiety mothers (Field et al., 2003). Postnatally the changes in systemic and cerebrovascular tone induced by prenatal stress are possibly also caused by changes in autonomic regulation since maternal stress and prenatal GC therapy are associated with changes in offspring's fetal heart rate and heart rate variability (Braeken et al., 2013; Lunshof et al., 2005; Monk et al., 2000, 2003, 2012; Mulder et al., 1994, 2004; Sjostrom et al., 2002). These results are in agreement with the overwhelming evidence from both animal and human studies that LBW, as a potential marker of prenatal stress, is associated with an altered autonomic function and hypertension in later life (for review see: (Phillips and Jones, 2006). It has been proposed that the changes in autonomic cardiovascular control are due to changes in baroreflex sensitivity (Jones et al., 2007).

Hypothalamus-pituitary-adrenocortical axis. Even though GC receptors (GR) are present in endothelial and VSMCs (Yang and Zhang, 2004), GCs do not directly regulate vascular tone. GC receptors act as transcription factors potentially influencing the expression of vasomediator agents such as norepinephrine and ANGII and their receptors on endothelial and VSMCs as well as the sensitivity of these receptors (Ullian, 1999; Xiao et al., 2003). Furthermore, cortisol seems to suppress the NO system which acts as a potent vasodilator (Kelly et al.,

1998). It is therefore biologically plausible that increased HPA axis activity after maternal stress and prenatal exposure to synthetic or endogenous GCs, as observed in humans and several animal species (for review see (Moisiadis and Matthews, 2014a,b), influences the function of the cardiovascular system. The extent to which changes in GR-sensitivity in the vasculature are involved in the programming of increased vascular tone needs to be examined in future studies.

4.3.2. Renin-angiotensin-aldosterone-system-mediated blood pressure regulation

Alterations in the renin-angiotensin-aldosterone-system (RAAS) programmed by prenatal exposure to synthetic GCs may also be responsible for marked increases in blood pressure in rats (O'Regan et al., 2004; Peers et al., 2001) and sheep (Dodic et al., 2002b; Moritz et al., 2002). The RAAS is an important regulator of blood pressure. Renin, produced in the kidney at low blood pressure, activates ANGII via a multistep process. ANGII binds to the two receptor subtypes AT_1 and AT_2 which are expressed in many tissues including vascular tissue. Whilst ANGII causes arterioles to constrict resulting in increased blood pressure, adrenals are stimulated to secrete aldosterone, a mineralocorticoid essential for fluid homeostasis and water retention. GCs acutely interfere with blood pressure regulation through the RAAS by binding to the mineralocorticoid receptor (MR) in the kidney. Fetal GC exposure during early gestation programs blood pressure regulation due to changes in kidney development and programming of mRNA expression of important components of the RAAS (for reviews see (McMillen and Robinson, 2005; Moritz et al., 2005; Nuyt, 2008; Wintour et al., 2003). Changes in kidney development result in a decreased nephron number and increased single nephron glomerular filtration rate in adult offspring (Moritz et al., 2011; Wintour et al., 2003). However, alterations in basal plasma concentrations of renin, angiotensinogen, angiotensin-I, angiotensin-converting-enzyme or ANGII could not be shown (Dodic et al., 2001; Peers et al., 2001), although the expression of AT_1 and AT_2 receptors in the brain and kidney was found to be increased (Dodic et al., 2006; Moritz et al., 2002). Thus, GC exposure in early gestation seems to program brain angiotensinergic circulatory control mechanisms which may contribute to programming of increased blood pressure in later life. (Dodic et al., 2006).

5. Conclusion and future directions

5.1. Gender effects

Human studies. There are very few human studies in the literature that address the effects of maternal stress or prenatal GC treatment on maternal-fetal circulation and programming of cardiovascular function with respect to the sex of the fetus. The association between maternal stress and an increase in umbilical resistance (Helbig et al., 2013) seems to be independent of the of the sex of the fetus although these effects of an association between maternal stress and an increase in uterine resistance with regard to a male/female fetus have not yet been studied. No sex-related effects of maternal stress or prenatal GC exposure on programming of cardiovascular function were found in the year-old (van Dijk et al., 2012) or in the 20- and 30-year-old offspring (Dalziel et al., 2005; Dessens et al., 2000). Due to the lack of sex-related studies with reference to maternal stress, we chose to review sex-specific effects of LBW as a proxy. LBW is a shared endpoint which may not only reflect prenatal undernutrition but can also be a result of maternal stress or prenatal GC treatment, probably because GCs stimulates organ maturation at the expense of cell division and, thus, growth (for review see (McMillen and Robinson, 2005). In a cohort of years-olds born with LBW but who were otherwise healthy, boys showed a higher blood pressure and systemic vascular resistance in the Trier Social Stress Test whereas girls showed an increased cardiac sympathetic activation (Jones et al., 2008). In line with this, a sex-specific impairment in autonomic blood pressure control at rest and during stress challenges was only found in young women who had LBW at birth (Jones et al., 2007).

Animal studies. In animal studies, slightly more attention has been paid to sex-specific differences in the programming of cardiovascular function although most studies were performed only in one sex. While experiments in rodents focused on male offspring (Banjanin et al., 2004; Kapoor and Matthews, 2005; Lesage et al., 2004; Levitt et al., 1996; Mastorci et al., 2009; Weinstock et al., 1998), female offspring were preferentially used in sheep models (Dodic et al., 2002a, 1998, 1999; Eckman et al., 2010; Wintour et al., 2003). Nevertheless, there is a growing body of evidence that prenatal stress programs cardiovascular function during later life in a sex-specific manner (Intapad et al., 2014; Fowden and Forhead, 2015). In male and female rat offspring, maternal stress did not change basal blood pressure but led to a prolonged blood pressure increase in response to acute restraint stress in female rat offspring (Igosheva et al., 2004). Prenatal GC exposure resulted in increased basal blood pressure in adolescent female but not male rats (O'Regan et al., 2004). After prenatal GC exposure, female rats also seem to be more susceptible to developing hypertension at a younger age whereas male rats develop it at an older age (Ortiz et al., 2003). This sex-specific effect could not be reproduced consistently in offspring of guinea pigs. While Liu et al. recorded no increased blood pressure in adult guinea pigs of both sexes, Banjanin et al. observed increased blood pressure in male offspring (Banjanin et al., 2004; Liu et al., 2001).

In spite of the inhomogeneous results of these few studies, sex differences in the programming of cardiovascular function by prenatal stress are probable since potential underlying mechanisms such as the RAAS and kidney development are modulated by sex hormones and synthetic GCs (Clifton and Murphy, 2004; Dasinger and Alexander, 2016; Intapad et al., 2014; Moritz et al., 2005). Further, GC-mediated programming of vascular function shows sex-specific characteristics. In sheep, prenatal GC exposure induces an increase in ET-1-mediated vascular resistance which is potentially a major mechanism for programming of vascular function (see Section 5.2) predominantly in female offspring (Lee et al., 2013). Apart from vascular dysfunction, programming of increased blood pressure by prenatal GC exposure seems to be associated with impaired renal function in male rats and sheep (Ortiz et al., 2003; Tang et al., 2010) and results in changes in the activity of the RAAS in female rats (O'Regan et al., 2004). No clear sex-dependent effects for programming of cardiovascular function have been shown, although sex-specific programming of the activity of the HPA axis as a potential mechanism of programming of cardiovascular function has been demonstrated (for review see (Dasinger and Alexander, 2016; Fowden and Forhead, 2015; Kapoor et al., 2006).

Taken together, the very limited data in human studies currently do not allow any firm conclusions to be drawn regarding a sex-specific association of maternal stress and prenatal GC exposure and programming of blood pressure in later life. In experimental studies, maternal stress and prenatal GC exposure seems to be associated with sex-specific differences for programming of blood pressure regulation in the offspring but study results vary broadly. Given the sex differences associated with stress sensitivity and resilience, the negligence of sex differences may contribute to the contradictory results achieved in human studies. More sex-related experimental and above all human studies are urgently needed.

5.2. Stress sensitive periods during pregnancy

Maternal stress in the form of maternal anxiety but not prenatal GC exposure seems to have adverse acute effects on maternal-fetal circulation throughout pregnancy. In the limited human studies available, ma-

ternal stress early during pregnancy appears to program offspring blood pressure (van Dijk et al., 2012). The effects of maternal stress during late gestation on cardiovascular function have not yet been examined in humans. The vulnerability of the fetus to maternal stress in early pregnancy is in agreement with vulnerable periods reported for the effects of malnutrition on cardiovascular programming (Painter et al., 2006, 2005a,b; Roseboom et al., 2006, 2011; van Dijk et al., 2012). Accordingly, data from sheep, the closest genetically-related species to humans examined in this regard to date, reveal that synthetic GC administration during early gestation (Dodic et al., 2001, 1998, 2006, 1999, 2002b; Peers et al., 2001; Wintour et al., 2003) and mid-gestation (Figueroa et al., 2005; Lee et al., 2013; Pulgar and Figueroa, 2006; Tang et al., 2010) programs increased blood pressure in juvenile and adult offspring. In rats, the programming effect of exposure to prenatal synthetic GCs on blood pressure has been examined only for exposure during the entire pregnancy or only for the last week of gestation (Celsi et al., 1998; Hadoke et al., 2006; Levitt et al., 1996; McDonald et al., 2003; O'Regan et al., 2004). Since the rat is an altricial species and rat pups are born very immature (compared to 7 mo in humans) (Dobbing and Sands, 1979; Romijn et al., 1991), translating results to the human situation with regard to the vulnerable periods of exposure to maternal stress or GCs is challenging. At the mechanistic level, maternal stress during early and mid-gestation but not during late gestation was accompanied by an extensive activation of the fetal HPA axis in term fetal sheep (Rakers et al., 2013). Moreover, GC exposure affects the development of fetal kidneys and the RAAS during early gestation (Dodic et al., 2001). During mid-to-late gestation, the GC-mediated programming of ET-1 induced-increase in vascular resistance becomes more prominent. In addition, predisposition to coronary heart disease may also be due to GC-induced programming of diminished NO-vasodilation in coronary arteries which occurs predominantly during early gestation.

Taken together, consistent experimental data suggest that early to mid-gestation is a vulnerable period for programming of increased blood pressure during later life. However, this blood pressure increase has not yet been reproduced in human studies. At best, although not reliably, human data on maternal stress indicate an increased resistance in uterine-placental circulation throughout pregnancy, which is consistent with experimental data. Clearly, additional and larger prospective human studies examining the effects of maternal stress and prenatal GC treatment are needed to determine the effects of maternal stress and GCs on post-natal cardiovascular function and to disclose vulnerable time windows during pregnancy.

5.3. Prenatal stress effects on maternal-fetal circulation – implications for brain development

To date, most human studies have examined the effects of maternal stress on maternal-fetal circulation. These studies are heterogeneous in design and outcome. They differ with regard to the maternal psychological variables such as anxiety, depression and psychological distress, the vascular bed of the maternal-fetal circulation as well as the Doppler indices used. Despite the inconsistencies, there is some evidence for an increase in vascular resistance in the uterine-placental circulation. In the sheep model, the acute increase in vascular resistance has been shown to be catecholamine-mediated (Rakers et al., 2015). Although the increase of uterine resistance in both human and sheep studies is moderate, it may influence fetal nutrient and oxygen supply and, thus, not only fetal growth (Brunton, 2013; Duthie and Reynolds, 2013; Frauendorf et al., 2011), but also cardiovascular and brain development. In humans, there is no evidence that the effects of maternal stress on maternal-fetal circulation are persistent. However, sheep studies show that the increase of uterine resistance during acute maternal stress is transient but secondary effects on fetal stress hormone release and fetal metabolism are lasting (Dreiling et al., 2016; Rakers et al., 2015). Moreover, chronic maternal stress induces long-term effects on uterine resistance (Dreiling et al., 2017). The assumption of a deficit in fetal nutrient and oxygen supply following a maternal stress -induced increase in uterine resistance is supported by an increase in placental size after maternal stress (Helbig et al., 2014; Tegethoff et al., 2010) which possibly reflects a compensatory adaptation to the disturbed maternal-fetal circulation. While a significant deficit in fetal nutrient and oxygen supply due to maternal stress has not yet been shown in humans, the maternal stress -induced decrease in uterine blood flow is accompanied by a prolonged shift to fetal lactacidosis in pregnant sheep (Dreiling et al., 2016; Rakers et al., 2015). However, it remains unclear whether it is the shift towards an anaerobic state or the potential deficit in fetal nutrient and oxygen supply due to maternal stress that reduces fetal growth causally. Elevated fetal cortisol and catecholamine levels may be responsible for the shift towards an anaerobic state in the fetus (Dreiling et al., 2017; Rakers et al., 2015) and directly affect cardiovascular and brain development due their tissue maturational effects at the expense of cell division (McMillen and Robinson, 2005; Weinstock, 2007; Wyrwoll and Holmes, 2012).

In fetal sheep, the increase of uterine resistance following maternal stress is paralleled by an increase in fetal blood pressure (Lohle et al., 2005; Schwab et al., 2000). After maternal administration of synthetic GCs, it has been shown that the increased fetal blood pressure is due to an increase in peripheral resistance and cardiac output (Jellyman et al., 2005; Schwab et al., 2006). The acute increase in peripheral vascular resistance is possibly due to an increased reactivity in response to receptor independent (potassium) and ET-1-mediated vasoconstriction (Anwar et al., 1999; Docherty et al., 2001). Some, but not all human studies, suggest that fetal cardiovascular adaption to maternal stress includes a compensatory redistribution of blood flow to the fetal brain induced by a decrease of cerebrovascular resistance (Malhotra et al., 2017b). Taking into account the fetal endogenous production of catecholamines during maternal stress in sheep (Rakers et al., 2015), the decrease of cerebrovascular resistance may be due to a lower density of alpha-adrenergic receptors in the cerebral circulation. But vasodilatory effects of acidosis may also decrease cerebrovascular resistance (Bevan et al., 1987; Dabertrand et al., 2012). However, it remains unclear to what extent the compensatory increase in cerebral blood flow can compensate for potential deficits in fetal nutrient and oxygen supply. Although the maternal stress -induced reduction in fetal growth seems to spare the brain in sheep (Frauendorf et al., 2011), fetal lactacidosis itself may affect neurodevelopment and cognitive and mental health outcomes (Eom and Lee, 2017).

Synthetic GCs appear to have differing effects on maternal-fetal circulation and, consequently, on the fetal brain compared to those induced by maternal stress. In both human and sheep studies, GC administration did not induce a prolonged alteration of uterine and umbilical blood flow possibly because synthetic GCs do not increase maternal and fetal catecholamines (Derks et al., 1997). Synthetic GCs acutely increase cerebrovascular resistance in fetal sheep with a consecutive decrease in cerebral blood flow (Lohle et al., 2005; Schütz et al., 1996; Schwab et al., 2000). The increase in cerebrovascular resistance might be due to the absence ofvasodilatory effects due to lactacidosis (Jellyman et al., 2005) which occurs during maternal stress (Dreiling et al., 2016; Dreiling et al., 2017; Rakers et al., 2015). In contrast, maternal anxiety and prenatal GC administration decrease the resistance of fetal MCA and the cerebro-umbilical ratio suggesting a potentially compensatory redistribution of blood flow to the fetal brain (Chitrit et al., 2000; Edwards et al., 2002; Muller et al., 2003; Piazze et al., 2001; Roos et al., 2015; Sjostrom et al., 1997; Urban et al., 2005). It does seem probable that the increase in cerebrovascular resistance has gone unnoticed in human studies since this effect appears to be transient (Lohle et al., 2005; Schwab et al., 2000). Moreover, in fetal sheep, cap-

illary cerebral blood flow was measured quantitatively using microspheres whilst, in human studies, blood flow in large brain feeding arteries, i.e. in arteries proximal to extraparenchymal and intracerebral arterioles that are the main regulators of cerebral blood flow (Faraci and Heistad, 1990), was measured using ultrasound Doppler sonography. Although the decrease in cerebral blood flow in fetal sheep was transient, decreased brain perfusion during GC exposure was associated with a transient loss of proteins involved in cerebral development and synaptogenesis (Antonow-Schlorke et al., 2009, 2007; Colberg et al., 2004; Schwab et al., 2001), and consequently, could have an effect on brain development and programming of mental and behavioral disorders.

Taken together, in spite of the adverse potential effects of maternal stress on maternal-fetal circulation and the consecutive changes in fetal metabolism, there are no data available as yet on the secondary effects of fetal brain development. Such effects are likely when one considers the high nutrient and oxygen demand of the developing brain (Gibbons, 1998) and the effects of disturbances in maternal-fetal circulation on fetal growth (Malhotra et al., 2017a) which is associated with disturbances in fetal brain development (Miller et al., 2016; Wang et al., 2016). Neurodevelopmental disturbances are potentially related to cognitive, behavioral and mental health problems in later life. The analysis of the effects of maternal stress on both fetal circulation and the secondary effects relating to brain development needs to be intensified in order to develop innovative targets for early prevention of altered cardiovascular and cognitive and mental health outcomes.

5.4. Prenatal stress effects on offspring circulation– implications for cognitive and mental health outcome

In contrast to several epidemiologic studies showing a link between LBW and the manifestation of cardiovascular diseases in later life (Alexander et al., 2015; Bruno et al., 2015; Roseboom et al., 2011), only a very small number of human studies have examined the effects of maternal stress on programming of cardiovascular function. If present at all, only modest associations between maternal stress and cardiovascular dysfunction during child- and adulthood are seen in the studies (Plana-Ripoll et al., 2016; van Dijk et al., 2012). Similarly, one study showed that prenatal administration of GCs to enhance fetal lung maturation results in a modest increase of blood pressure during adolescence (Doyle et al., 2000). However, studies comparing single vs. multiple doses of BM couldn't show an effect of increased dosage (Dalziel et al., 2007; Dessens et al., 2000). In contrast, animal experiments in rodents and sheep showed programming of cardiovascular function in offspring after maternal stress (Mastorci et al., 2009; Roussel et al., 2004). This effect was even more pronounced after prenatal GC administration (Dodic et al., 1998; Hadoke et al., 2006). Programming effects included increased blood pressure, left ventricular hypertrophy and an increase in cardiac output in adulthood. In the face of the reproducible evidence in different animal species and evidence of human intra-uterine growth retardation (Bassareo et al., 2016; Rasyid and Bakri, 2016), it is not very plausible that maternal stress and/or prenatal GC treatment have no programming effects on cardiovascular function in humans. The great variety of prenatal and postnatal influences in humans, e.g. preterm labour following prenatal BM treatment may modulate the effects of maternal stress or prenatal GC exposure. Thus, detecting programming effects in the relatively small prospective cohort studies is a challenging task. Moreover, the programming effects of maternal stress or prenatal GC treatment on cardiovascular function may not manifest themselves until older age when additional cardiovascular risk factors occur and cardiac function and vascular reactivity decrease physiologically.

At the mechanistic level, programming of enhanced sympathetic and HPA axis activity, impaired kidney development and central re-setting of the RAAS, as well as the changes in vascular reactivity and structure may all play a role in altered blood pressure regulation. Among the different vasomediators involved in peripheral resistance regulation, the vasoconstrictor ET-1 system is most vulnerable to programming effects of prenatal GC-exposure. Similar to the peripheral resistance vessels, coronary vascular reactivity in sheep shows persisting changes after prenatal GC exposure. The most prominent GC effect comprises a diminished vasodilator response to NO (Roghair et al., 2004, 2005a). The decrease in coronary artery vasodilator capacity may amplify the negative effects of atherosclerosis on the development of cardiovascular diseases associated with prenatal GC exposure. At the structural level, increased arterial stiffness has been suggested as being a potential mechanism for the increased blood pressure in LBW offspring but a direct study of the effects of maternal stress and prenatal GC exposure has to date not been undertaken (Leeson et al., 1997; Wilkinson and Cockcroft, 2004). Prenatal GC exposure during late gestation also increased α-actin content in fetal sheep arteries which may be a major contributor to arterial stiffness in later life (Matuszek et al., 2006). Interestingly, arterial stiffness is a good predictor of cognitive impairment (Waldstein et al., 2008). Arterial stiffness impairs cerebrovascular autoregulation, i.e. vasoconstriction and vasodilation of pial and intracerebral arteries and arterioles, to adjust vascular tone in order to maintain a relatively constant cerebral blood flow within a range of pressures (Iadecola, 2013). Disturbances in cerebral autoregulation potentially lead to altered oxygen and nutrient supply to the brain when the blood pressure decreases. In addition, altered cerebral autoregulation makes the brain also vulnerable to the effects of blood pressure peaks which may induce microbleedings (Iadecola and Davisson, 2008; Nagai et al., 2010). Both may contribute to cognitive impairment. However, neither the experimental nor the human studies explored the effects of maternal stress or prenatal GC exposure on cerebrovascular function during later life.

The effects of endothelial dysfunction and arterial stiffness on cognitive outcome most likely occur at an older age. However, neither human nor experimental studies on the cardiovascular or cognitive and mental health outcome have been undertaken to date. An increase in arterial stiffness is a characteristic of vascular aging (Pitale and Sahasrabuddhe, 2011). The vascular endothelium undergoes a transition from an anti-atherosclerotic state to a pro-atherosclerotic state accompanied by structural changes of the arterial vessel wall which is additionally associated with impaired endothelial-mediated vasodilation. In the presence of arterial hypertension, which is probably the predominant functional cardiovascular outcome parameter programmed by adverse intrauterine conditions (Bassareo et al., 2016; Rasyid and Bakri, 2016), age-associated vascular, cognitive and structural abnormalities in the brain including brain atrophy occur prematurely (Baumbach and Heistad, 1988; Jennings and Zanstra, 2009; Lane and Wager, 2009; Raz and Rodrigue, 2006). However, to date there are no human or experimental studies that have examined the effects of maternal stress or prenatal GC treatment on vascular and brain aging. This should definitely constitute a future avenue of research.

Arterial hypertension fuels atherosclerosis and arterial stiffening, impairs cerebrovascular reactivity and causes regional reduction of cerebral blood flow (Iadecola and Davisson, 2008). These effects constitute risk factors for classical cerebrovascular diseases such as microvascular damage, white matter lesions, ischemic stroke, and intracranial hemorrhage (Iadecola and Davisson, 2008). Currently there are no clinical data available on a potential relationship between prenatal stress and the incidence of cerebrovascular disease. In two experimental studies, adult rats exposed to prenatal but not neonatal stress showed increased neurological deficits, infarct size and apoptosis after middle cerebral artery occlusion (Hays et al., 2013; Wang et al., 2015). In the face of the increasing prevalence of cerebrovascular diseases, more studies are needed on the programming effects of prenatal stress

on cardiovascular function since they provide a strong potential for preventive and interventional measures for cognitive and mental health disorders.

6. Overall summary

The inconsistent evidence available suggests at best that maternal stress in the form of maternal anxiety increases uterine resistance. The secondary decrease in nutrient and oxygen supply potentially affects neurodevelopment but this has not yet been shown and should be a focus of future research. In contrast, there is very limited evidence in humans on the programming effects of maternal stress on offspring cardiovascular function in later life. A moderately increased risk for cardiovascular diseases was nevertheless found in adults whose mothers experienced severe psychological stress during pregnancy. The scarce data on the relationship between maternal stress or prenatal GC treatment and cardiovascular dysfunction account for the fact that the potential role of cardiovascular dysfunction programmed by maternal stress or prenatal GC exposure on cerebrovascular, cognitive and mental health outcome cannot be reliably evaluated. Nevertheless, such a relationship seems to be possible since a number of retrospective human studies show an association between LBW and cardiovascular dysfunction or cognitive and mental health problems in later life (Mathewson et al., 2017; Simeoni and Zetterstrom, 2005; Vaiserman, 2018). However, these studies did not account for the cause of LBW i.e. whether it was due to prenatal malnutrition or associated stress. Moreover, the link between cardiovascular dysfunction and cognitive and mental health problems was not examined in these studies. Hence, much of the evidence on cardiovascular programming following maternal stress has been derived from animal models after prenatal GC exposure. Prenatal GCs induce structural alterations of the vasculature and alterations in blood pressure regulation as well as changes in peripheral and coronary but not cerebrovascular reactivity. Of course, animal studies only represent models that do not replace human studies. But they have the advantage of introducing standardized stress, exploring mechanisms and providing indications for endpoints in human studies. There is definitely a need for prospective longitudinal experimental and human studies examining the effects of prenatal stress on offspring cardiovascular outcome and its association to cerebrovascular, cognitive and mental health outcome. In addition, measuring basal blood pressure is probably not the most sensitive method to detect fine differences in vascular function in cohorts of young subjects. Although the effects of arterial hypertension constitute vascular risk factors for age-related cognitive decline and mild cognitive impairment, which is a transition phase between healthy cognitive aging and dementia (Iadecola and Davisson, 2008; Nagai et al., 2010), no experimental or human studies have examined the effects of maternal stress or prenatal GC exposure on cardiovascular function during aging when the effects of prenatal stress are more likely to appear. This is foremost a field for experimental studies, since prospective studies in humans until old age are less feasible. It is important to examine cardiovascular and cerebrovascular function under challenge and to explore the concept of resilience. Sex-specific differences should also be a focus in future research approaches in animal as well as in human studies. Forthcoming experimental studies should also investigate whether therapeutic targeting of cardiovascular dysfunction can influence cognitive and mental health outcome and whether findings can be translated to humans. Until such studies are undertaken, the conclusion of Mastorci et al. (2009) from almost 10 years ago is still valid: prenatal stress by itself does not appear to change a given structure or function dramatically but it affects resilience and renders the individual more susceptible to further insults occurring in adulthood.

Acknowledgement

The authors received support from the EU FP7/Health.2011.2.22-2 GA 279281.

References

Ahima, R., Krozowski, Z., Harlan, R.E., 1991. Type I corticosteroid receptor-like immunoreactivity in the rat CNS: distribution and regulation by corticosteroids. J. Comp. Neurol. 313, 522–538.
Alexander, B.T., Dasinger, J.H., Intapad, S., 2015. Fetal programming and cardiovascular pathology. Compr. Physiol. 5, 997–1025.
Antonow-Schlorke, I., Helgert, A., Gey, C.T., Coksaygan, T., Schubert, H., Nathanielsz, P.W., Witte, O.W., Schwab, M., 2009. Adverse effects of antenatal glucocorticoids on cerebral myelination in sheep. Obstet. Gynecol. 113, 142–151.
Antonow-Schlorke, I., Muller, T., Brodhun, M., Wicher, G., Schubert, H., Nathanielsz, P.W., Witte, O.W., Schwab, M., 2007. Betamethasone-related acute alterations of microtubule-associated proteins in the fetal sheep brain are reversible and independent of age during the last one-third of gestation. Am. J. Obstet. Gynecol. 196 (553), e551–e553, e556.
Anwar, M.A., Saleh, A.I., Al Olabi, R., Al Shehabi, T.S., Eid, A.H., 2016. Glucocorticoid-induced fetal origins of adult hypertension: association with epigenetic events. Vasc. Pharmacol. 82, 41–50.
Anwar, M.A., Schwab, M., Poston, L., Nathanielsz, P.W., 1999. Betamethasone-mediated vascular dysfunction and changes in hematological profile in the ovine fetus. Am. J. Physiol.-Heart C 276, H1137–H1143.
Banjanin, S., Kapoor, A., Matthews, S.G., 2004. Prenatal glucocorticoid exposure alters hypothalamic-pituitary-adrenal function and blood pressure in mature male guinea pigs. J. Physiol.-Lond. 558, 305–318.
Barkehall-Thomas, A., Thompson, M., Baker, L.S., Edwards, A., Wallace, E.M., 2003. Betamethasone associated changes in umbilical artery flow velocity waveforms in multiple pregnancies with umbilical artery absent end diastolic flow. Aust. Nz. J. Obstet. Gyn. 43, 360–363.
Bartha, J.L., Largo-Heinrich, M., Machado, M.J., Gonzalez-Bugatto, F., Hervias-Vivancos, B., 2008. Effects of antenatal betamethasone on human fetal branch pulmonary artery flow velocity waveforms. Fetal Diagn. Ther. 23, 46–53.
Bassareo, P.P., Marras, A.R., Cugusi, L., Zedda, A.M., Mercuro, G., 2016. The reasons why cardiologists should consider prematurity at birth and intrauterine growth retardation among risk factors. J. Cardiovasc. Med. 17, 323–329.
Baumbach, G.L., Heistad, D.D., 1988. Cerebral-circulation in chronic arterial-hypertension.. Hypertension 12, 89–95.
Bell, R.D., Winkler, E.A., Sagare, A.P., Singh, I., LaRue, B., Deane, R., Zlokovic, B.V., 2010. Pericytes control key neurovascular functions and neuronal phenotype in the adult brain and during brain aging. Neuron 68, 409–427.
Bennet, L., Kozuma, S., McGarrigle, H.H.G., Hanson, M.A., 1999. Temporal changes in fetal cardiovascular, behavioural, metabolic and endocrine responses to maternally administered dexamethasone in the late gestation fetal sheep. Brit. J. Obstet. Gynaecol. 106, 331–339.
Berry, L.M., Polk, D.H., Ikegami, M., Jobe, A.H., Padbury, J.F., Ervin, M.G., 1997. Preterm newborn lamb renal and cardiovascular responses after fetal or maternal antenatal betamethasone. Am. J. Physiol.-Regul. I 272, R1972–R1979.
Bevan, J.A., Bevan, R.D., 1981. Developmental influences on vascular structure and function. Ciba Found. Symp. 83, 94–107.
Bevan, J.A., Duckworth, J., Laher, I., Oriowo, M., McPherson, G., Bevan, R., 1987. Sympathetic control of cerebral arteries: specialization in receptor type, reserve, affinity, and distribution. FASEB J. 1, 193–198.
Blencowe, H., Cousens, S., Oestergaard, M.Z., Chou, D., Moller, A.B., Narwal, R., Adler, A., Garcia, C.V., Rohde, S., Say, L., Lawn, J.E., 2012. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet 379, 2162–2172.
Blood, A.B., Zhao, Y., Long, W., Zhang, L., Longo, L.D., 2002. L-type Ca2+ channels in fetal and adult ovine cerebral arteries. Am. J. Physiol.-Reg. I 282, R131–R138.
Bock, J., Wainstock, T., Braun, K., Segal, M., 2015. Stress in utero: prenatal programming of brain plasticity and cognition. Biol. Psychiat. 78, 315–326.
Boesveld, M., Heida, K.Y., Oudijk, M.A., Brouwers, H.A.A., Koenen, S.V., Kwee, A., 2014. Evaluation of antenatal corticosteroid prescribing patterns among 984 women at risk for preterm delivery. J. Matern.-Fetal Neon. Med. 27, 516–519.
Braeken, M.A.K.A., Kemp, A.H., Outhred, T., Otte, R.A., Monsieur, G.J.Y.J., Jones, A., Van den Bergh, B.R.H., 2013. Pregnant mothers with resolved anxiety disorders and their offspring have reduced heart rate variability: implications for the health of children. Plos One 8.
Brown, R.W., Diaz, R., Robson, A.C., Kotelevtsev, Y.V., Mullins, J.J., Kaufman, M.H., Seckl, J.R., 1996. The ontogeny of 11 beta-hydroxysteroid dehydrogenase type 2 and mineralocorticoid receptor gene expression reveal intricate control of glucocorticoid action in development. Endocrinology 137, 794–797.
Bruno, R.M., Faconti, L., Taddei, S., Ghiadoni, L., 2015. Birth weight and arterial hypertension. Curr. Opin. Cardiol. 30, 398–402.
Brunton, P., 2015. Programming the brain and behaviour by early-life stress: a focus on neuroactive steroids. J. Neuroendocrinol. 27, 468–480.

Brunton, P.J., 2013. Effects of maternal exposure to social stress during pregnancy: consequences for mother and offspring. Reproduction 146, R175–R189.
Çalışkan, E., Özkan, S., Çakıroğlu, Y., Yalçınkaya, O., Polat, A., Çorakçı, A., 2009. The effects of maternal anxiety prior to amniocentesis on uterine and fetal umbilical blood flow. J. Turk. Ger. Gynecol. Assoc. 10, 162–167.
Gelsi, G., Kistner, A., Aizman, R., Eklof, A.C., Ceccatelli, S., De Santiago, A., Jacobson, S.H., 1998. Prenatal dexamethasone causes oligonephronia, sodium retention, and higher blood pressure in the offspring. Pediatr. Res. 44, 317–322.
Chandrasekaran, S., Srinivas, S.K., 2014. Antenatal corticosteroid administration: understanding its use as an obstetric quality metric. Am. J. Obstet. Gynecol. 210.
Chern, J., Kamm, K.E., Rosenfeld, C.R., 1995. Smooth-muscle myosin heavy-chain isoforms are developmentally-regulated in male fetal and neonatal sheep. Pediatr. Res. 38, 697–703.
Chitrit, Y., Caubel, P., Herrero, R., Schwinte, A.L., Guillaumin, D., Boulanger, M.C., 2000. Effects of maternal dexamethasone administration on fetal Doppler flow velocity waveforms. Brit. J. Obstet. Gynaecol. 107, 501–507.
Clifton, V., Murphy, V., 2004. Maternal asthma as a model for examining fetal sex-specific effects on maternal physiology and placental mechanisms that regulate human fetal growth. Placenta 25, S45–S52.
Cohlen, B.J., Stigter, R.H., Derks, J.B., Mulder, E.J.H., Visser, G.H.A., 1996. Absence of significant hemodynamic changes in the fetus following maternal betamethasone administration. Ultrasound Obst. Gynocol. 8, 252–255.
Colberg, C., Antonow-Schlorke, I., Müller, T., Schubert, H., Witte, O.W., Schwab, M., 2004. Recovery of glucocorticoid-related loss of synaptic density in the fetal sheep brain at 0.75 of gestation. Neurosci. Lett. 364, 130–134.
Corner, R.A., Kenyon, P.R., Stafford, K.J., West, D.M., Oliver, M.H., 2010. The effect of different types of stressors during mid- and late pregnancy on lamb weight and body size at birth. Animal 4, 2065–2070.
Crossley, K.J., Morley, C.J., Allison, B.J., Davis, P.G., Polglase, G.R., Wallace, M.J., Zahra, V.A., Hooper, S.B., 2009. Antenatal corticosteroids increase fetal, but not postnatal, pulmonary blood flow in sheep. Pediatr. Res. 66, 283–288.
Dabertrand, F., Nelson, M.T., Brayden, J.E., 2012. Acidosis dilates brain parenchymal arterioles by conversion of calcium waves to sparks to activate BK channels novelty and significance. Circ. Res. 110, 285–294.
Dalziel, S.R., Parag, V., Rodgers, A., Harding, J.E., 2007. Cardiovascular risk factors at age 30 following preterm birth. Early Hum. Dev. 83, S51–S52.
Dalziel, S.R., Walker, N.K., Parag, V., Martell, G., Rea, H.H., Rodgers, A., Harding, J.E., 2005. Cardiovascular risk factors after antenatal exposure to betamethasone: 30-year follow-up of a randomised controlled trial. Lancet 365, 1856–1862.
Dampney, R.A.L., 1994. Functional-organization of Central pathways regulating the cardiovascular-system. Physiol. Rev. 74, 323–364.
Dasinger, J.H., Alexander, B.T., 2016. Gender differences in developmental programming of cardiovascular diseases. Clin. Sci. 130, 337–348.
Deren, O., Karaer, C., Onderoglu, L., Yigit, N., Durukan, T., Bahado-Singh, R.O., 2001. The effect of steroids on the biophysical profile and Doppler indices of umbilical and middle cerebral arteries in healthy preterm fetuses. Eur J. Obst. Gynecol. Rep. Biol. 99, 72–76.
Derks, J.B., Giussani, D.A., Jenkins, S.L., Wentworth, R.A., Visser, G.H.A., Padbury, J.F., Nathanielsz, P.W., 1997. A comparative study of cardiovascular, endocrine and behavioural effects of betamethasone and dexamethasone administration to fetal sheep. J. Physiol.-Lond. 499, 217–226.
Dessens, A.B., de Haas, H.S., Koppe, J.G., 2000. Twenty-year follow-up of antenatal corticosteroid treatment. Pediatrics 105.
Dobbing, J., Sands, J., 1979. Comparative aspects of the brain growth spurt. Early Hum. Dev. 3, 79–83.
Docherty, C.C., Kalmar-Nagy, J., Engelen, M., Koenen, S.V., Nijland, M., Kuc, R.E., Davenport, A.P., Nathanielsz, P.W., 2001. Effect of in vivo fetal infusion of dexamethasone at 0.75 GA on fetal ovine resistance artery responses to ET-1. Am. J. Physiol.-Reg. I 281, R261–R268.
Dodic, M., Baird, R., Hantzis, V., Koukoulas, I., Moritz, K., Peers, A., Wintour, E.M., 2001. Organs/systems potentially involved in one model of programmed hypertension in sheep. Clin. Exp. Pharmacol. Physiol. 28, 952–956.
Dodic, M., Hantzis, V., Duncan, J., Rees, S., Koukoulas, I., Johnson, K., Wintour, E.M., Moritz, K., 2002. Programming effects of short prenatal exposure to cortisol. Faseb J. 16, 1017–1026.
Dodic, M., May, C.N., Wintour, E.M., Coghlan, J.P., 1998. An early prenatal exposure to excess glucocorticoid leads to hypertensive offspring in sheep. Clin. Sci. 94, 149–155.
Dodic, M., McAlinden, A.T., Jefferies, A.J., Wintour, E.M., Cock, M.L., May, C.N., Evans, R.G., Moritz, K.M., 2006. Differential effects of prenatal exposure to dexamethasone or cortisol on circulatory control mechanisms mediated by angiotensin II in the central nervous system of adult sheep. J. Physiol.-Lond. 571, 651–660.
Dodic, M., Peers, A., Coghlan, J.P., May, C.N., Lumbers, E., Yu, Z.Y., Wintour, E.M., 1999. Altered cardiovascular haemodynamics and baroreceptor-heart rate reflex in adult sheep after prenatal exposure to dexamethasone. Clin. Sci. 97, 103–109.
Dodic, M., Peers, A., Moritz, K., Hantzis, V., Wintour, E.M., 2002. No evidence for HPA reset in adult sheep with high blood pressure due to short prenatal exposure to dexamethasone. Am. J. Physiol.-Reg. Integ. 282, R343–R350.
Dodic, M., Wintour, E.M., 1994. Effects of prolonged (48-h) infusion of cortisol on blood-pressure, renal-function and fetal fluids in the immature ovine fetus. Clin. Exp. Pharmacol. Physiol. 21, 971–980.
Doyle, L.W., Ford, G.W., Davis, N.M., Callanan, C., 2000. Antenatal corticosteroid therapy and blood pressure at 14 years of age in preterm children. Clin. Sci. 98, 137–142.
Dreiling, M., Bischoff, S., Schiffner, R., Rupprecht, S., Kiehntopf, M., Schubert, H., Witte, O.W., Nathanielsz, P.W., Schwab, M., Rakers, F., 2016. Stress-induced decrease of uterine blood flow in sheep is mediated by alpha 1-adrenergic receptors. Stress 19, 547–551.
Dreiling, M., Schiffner, R., Bischoff, S., Rupprecht, S., Kroegel, N., Schubert, H., Witte, O.W., Schwab, M., Rakers, F., 2017. Impact of chronic maternal stress during early gestation on maternal–fetal stress transfer and fetal stress sensitivity in sheep. Stress 1–10.
Duthie, L., Reynolds, R.M., 2013. Changes in the maternal hypothalamic-pituitary-adrenal axis in pregnancy and postpartum: influences on maternal and fetal outcomes. Neuroendocrinology 98, 106–115.
Dyer, J.S., Rosenfeld, C.R., 2011. Metabolic imprinting by prenatal, perinatal, and postnatal overnutrition: a review. Semin. Reprod. Med. 29, 266–276.
Eckman, D.M., Kerr, B.A., Puloria, M., Simandle, S.A., Watt, S.E., Rose, J.C., Figueroa, J.P., 2010. Antenatal betamethasone alters vascular reactivity in adult female ovine cerebral arteries. Pediatr. Res. 68, 344–348.
Edwards, A., Baker, L.S., Wallace, E.M., 2002. Changes in fetoplacental vessel flow velocity waveforms following maternal administration of betamethasone. Ultrasound Obst. Gyn. 20, 240–244.
Edwards, A., Baker, L.S., Wallace, E.M., 2003. Changes in umbilical artery flow velocity waveforms following maternal administration of betamethasone. Placenta 24, 12–16.
Eglen, R.M., Whiting, R.L., 1990. Heterogeneity of vascular muscarinic receptors. J. Auton. Pharmacol. 10, 233–245.
Eom, S., Lee, Y.-M., 2017. Preliminary study of neurodevelopmental outcomes and parenting stress in pediatric mitochondrial disease. Pediatr. Neurol. 71, 43–49, e41.
Faraci, F.M., Heistad, D.D., 1990. Regulation of large cerebral-arteries and cerebral microvascular pressure. Circ. Res. 66, 8–17.
Field, T., Diego, M., Hernandez-Reif, M., Schanberg, S., Kuhn, C., Yando, R., Bendel, D., 2003. Pregnancy anxiety and comorbid depression and anger: effects on the fetus and neonate. Depress. Anxiety 17, 140–151.
Figueroa, J.P., Rose, J.C., Massmann, G.A., Zhang, J., Acuna, G., 2005. Alterations in fetal kidney development and elevations in arterial blood pressure in young adult sheep after clinical doses of antenatal glucocorticoids. Pediatr. Res. 58, 510–515.
Fletcher, A.J.W., McGarrigle, H.H.G., Edwards, C.M.B., Fowden, A.L., Giussani, D.A., 2002. Effects of low dose dexamethasone treatment on basal cardiovascular and endocrine function in fetal sheep during late gestation. J. Physiol.-Lond. 545, 649–660.
Fowden, A.L., Forhead, A.J., 2015. Glucocorticoids as regulatory signals during intrauterine development. Exp. Physiol. 100, 1477–1487.
Furchgott, R.F., Zawadzki, J.V., 1980. The obligatory role of endothelial-cells in the relaxation of arterial smooth-muscle by acetylcholine. Nature 288, 373–376.
Gibbons, A., 1998. Solving the brain's energy crisis. Science 280, 1345–1347.
Gitau, R., Fisk, N., Glover, V., 2001. Maternal stress in pregnancy and its effect on the human foetus: an overview of research findings. Stress 4, 195–203.
Gomez, O., Figueras, F., Fernandez, S., Bennasar, M., Martinez, J.M., Puerto, B., Gratacos, E., 2008. Reference ranges for uterine artery mean pulsatility index at 11-41 weeks of gestation. Ultrasound Obstet. Gynecol. 32, 128–132.
Hadoke, P.W.F., Lindsay, R.S., Seckl, J.R., Walker, B.R., Kenyon, C.J., 2006. Altered vascular contractility in adult female rats with hypertension programmed by prenatal glucocorticoid exposure. J. Endocrinol. 188, 435–442.
Hai, C.M., Sadowska, G., Francois, L., Stonestreet, B.S., 2002. Maternal dexamethasone treatment alters myosin isoform expression and contractile dynamics in fetal arteries. Am. J. Physiol.-Heart Circul. 283, H1743–H1749.
Harris, A., Seckl, J., 2011. Glucocorticoids, prenatal stress and the programming of disease. Horm. Behav. 59, 279–289.
Harville, E.W., Savitz, D.A., Dole, N., Herring, A.H., Thorp, J.M., Light, K.C., 2008. Stress and placental resistance measured by Doppler ultrasound in early and mid-pregnancy. Ultrasound Obst. Gyn. 32, 23–30.
Hassan, Av., Von Rosenstiel, P., Patchev, V., Holsboer, F., Almeida, O., 1996. Exacerbation of apoptosis in the dentate gyrus of the aged rat by dexamethasone and the protective role of corticosterone. Expe. Neurol. 140, 43–52.
Haynes, W.G., Webb, D.J., 1994. Contribution of endogenous generation of endothelin-1 to basal vascular tone. Lancet 344, 852–854.
Hays, S.L., Valieva, O.A., McPherson, R.J., Juul, S.E., Gleason, C.A., 2013. Adult responses to an ischemic stroke in a rat model of neonatal stress and morphine treatment. Int. J. Dev. Neurosci. 31, 25–29.
Helbig, A., Kaasen, A., Malt, U.F., Haugen, G., 2011. Psychological distress after recent detection of fetal malformation: short-term effect on second-trimester uteroplacental and fetoplacental circulation. BJOG-Int. J. Obstet. Gyanecol. 118, 1653–1657.
Helbig, A., Kaasen, A., Malt, U.F., Haugen, G., 2013. Does antenatal maternal psychological distress affect placental circulation in the third trimester?. Plos One 8.
Helbig, A., Kaasen, A., Malt, U.F., Haugen, G., 2014. Maternal psychological distress and placental circulation in pregnancies after a previous offspring with congenital malformation. Plos One 9.
Hu, F., Morrissey, P., Yao, J., Xu, Z., 2004. Development of AT 1 and AT 2 receptors in the ovine fetal brain. Dev. Brain Res. 150, 51–61.
Hutanu, C., Cox, B.E., DeSpain, K., Liu, X.T., Rosenfeld, C.R., 2007. Vascular development in early ovine gestation: carotid smooth muscle function, phenotype, and biochemical markers. Am. J. Physiol.-Regul. Integer. 293, R323–R333.
Iadecola, C., 2013. The pathobiology of vascular dementia. Neuron 80, 844–866.
Iadecola, C., Davisson, R.L., 2008. Hypertension and cerebrovascular dysfunction. Cell Metab. 7, 476–484.
Igosheva, N., Klimova, O., Anishchenko, T., Glover, V., 2004. Prenatal stress alters cardiovascular responses in adult rats. J. Physiol.-Lond. 557, 273–285.

Igosheva, N., Taylor, P.D., Poston, L., Glover, V., 2007. Prenatal stress in the rat results in increased blood pressure responsiveness to stress and enhanced arterial reactivity to neuropeptide Y in adulthood. J. Physiol.-Lond. 582, 665–674.
Intapad, S., Ojeda, N.B., Dasinger, J.H., Alexander, B.T., 2014. Sex differences in the developmental origins of cardiovascular disease. Physiology 29, 122–132.
Järvelin, M.-R., Sovio, U., King, V., Lauren, L., Xu, B., McCarthy, M.I., Hartikainen, A.-L., Laitinen, J., Zitting, P., Rantakallio, P., 2004. Early life factors and blood pressure at age 31 years in the 1966 northern Finland birth cohort. Hypertension 44, 838–846.
Jellyman, J., Gardner, D., Edwards, C., Fowden, A., Giussani, D., 2005. Fetal cardiovascular, metabolic and endocrine responses to acute hypoxaemia during and following maternal treatment with dexamethasone in sheep. J. Physiol. 567, 673–688.
Jennings, J.R., Zanstra, Y., 2009. Is the brain the essential in hypertension?. Neuroimage 47, 914–921.
Jones, A., Beda, A., Osmond, C., Godfrey, K.M., Simpson, D.M., Phillips, D.I.W., 2008. Sex-specific programming of cardiovascular physiology in children. Eur. Heart J. 29, 2164–2170.
Jones, A., Beda, A., Ward, A.M.V., Osmond, C., Phillips, D.I.W., Moore, V.M., Simpson, D.M., 2007. Size at birth and autonomic function during psychological stress. Hypertension 49, 548–555.
Kähler, C., Schleussner, E., Möller, A., Seewald, H.J., 2004. Doppler measurements in feto-placental vessels after maternal betamethasone administration. Fetal Diagn. Ther. 19, 52–57.
Kaiser, S., Schwerdt, B., Siegeler, K., Sachser, N., 2015. Social instability during pregnancy and lactation alters female wild cavy offsprings' endocrine status and behaviour later in life. Behaviour 152, 837–859.
Kalaria, R.N., 2010. Vascular basis for brain degeneration: faltering controls and risk factors for dementia. Nutr. Rev. 68, S74–S87.
Kapoor, A., Dunn, E., Kostaki, A., Andrews, M.H., Matthews, S.G., 2006. Fetal programming of hypothalamo-pituitary-adrenal function: prenatal stress and glucocorticoids. J. Physiol.-Lond. 572, 31–44.
Kapoor, A., Matthews, S.G., 2005. Short periods of prenatal stress affect growth, behaviour and hypothalamo-pituitary-adrenal axis activity in male guinea pig offspring. J. Physiol.-Lond. 566, 967–977.
Karaki, H., Urakawa, N., Kutsky, P., 1984. Potassium-induced contraction in smooth muscle. ????????? 20, 427–444.
Kelly, J.J., Mangos, G., Williamson, P.M., Whitworth, J.A., 1998. Cortisol and hypertension. Clin. Exp. Pharmacol. Physiol. 25, S51–S56.
Kent, A., Hughes, P., Ormerod, L., Jones, G., Thilaganathan, B., 2002. Uterine artery resistance and anxiety in the second trimester of pregnancy. Ultrasound Obst. Gyn. 19, 177–179.
Kliewer, S.A., Moore, J.T., Wade, L., Staudinger, J.L., Watson, M.A., Jones, S.A., McKee, D.D., Oliver, B.B., Willson, T.M., Zetterström, R.H., 1998. An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. Cell 92, 73–82.
Koenen, S.V., Mecenas, C.A., Smith, G.S., Jenkins, S., Nathanielsz, P.W., 2002. Effects of maternal betamethasone administration on fetal and maternal blood pressure and heart rate in the baboon at 0.7 of gestation. Am. J. Obstet. Gynecol. 186, 812–817.
Lane, R.D., Wager, T.D., 2009. Introduction to a Special issue of neuroimage on brain-body medicine. Neuroimage 47, 781–784.
Lee, H.C., Lyndon, A., Blumenfeld, Y.J., Dudley, R.A., Gould, J.B., 2011. Antenatal steroid administration for premature neonates in California. Obstet. Gynecol. 117, 603–609.
Lee, J.H., Zhang, J., Flores, L., Rose, J.C., Massmann, G.A., Figueroa, J.P., 2013. Antenatal betamethasone has a sex-dependent effect on the in vivo response to endothelin in adult sheep. Am. J. Physiol.-Reg. Integer. 304, R581–R587.
Lee, J.H., Zhang, J., Massmann, G.A., Figueroa, J.P., 2014. Antenatal betamethasone increases vascular reactivity to endothelin-1 by upregulation of CD38/cADPR signaling. J. Dev. Orig. Health Dis. 5, 56–62.
Leeson, C.P.M., Whincup, P.H., Cook, D.G., Donald, A.E., Papacosta, O., Lucas, A., Deanfield, J.E., 1997. Flow-mediated dilation in 9- to 11-year-old children - the influence of intrauterine and childhood factors. Circulation 96, 2233–2238.
Lesage, J., Del-Favero, F., Leonhardt, M., Louvart, H., Maccari, S., Vieau, D., Darnaudery, M., 2004. Prenatal stress induces intrauterine growth restriction and programmes glucose intolerance and feeding behaviour disturbances in the aged rat. J. Endocrinol. 181, 291–296.
Levitt, N.S., Lindsay, R.S., Holmes, M.C., Seckl, J.R., 1996. Dexamethasone in the last week of pregnancy attenuates hippocampal glucocorticoid receptor gene expression and elevates blood pressure in the adult offspring in the rat. Neuroendocrinology 64, 412–418.
Li, B.W., Freeman, R.D., 2010. Neurometabolic coupling in the lateral geniculate nucleus changes with extended age. J. Neurophysiol. 104, 414–425.
Liu, L., Li, A.T., Matthews, S.G., 2001. Maternal glucocorticoid treatment programs HPA regulation in adult offspring: sex-specific effects. Am. J. Physiol.-Endocrinol. Metab. 280, E729–E739.
Lohle, M., Muller, T., Wicher, C., Roedel, M., Schubert, H., Witte, O.W., Nathanielsz, P.W., Schwab, M., 2005. Betamethasone effects on fetal sheep cerebral blood flow are not dependent on maturation of cerebrovascular system and pituitary-adrenal axis. J. Physiol.-Lond. 564, 575–588.
Ludmer, P.L., Selwyn, A.P., Shook, T.L., Wayne, R.R., Mudge, G.H., Alexander, R.W., Ganz, P., 1986. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. New Engl. J. Med. 315, 1046–1051.
Lunshof, M.S., Boer, K., Wolf, H., Koppen, S., Velderman, J.K., Mulder, E.J.H., 2005. Short-term (0–48 h) effects of maternal betamethasone administration on fetal heart rate and its variability. Pediatr. Res. 57, 545–549.
Maina, G., Saracco, P., Giolito, M.R., Danelon, D., Bogetto, F., Todros, T., 2008. Impact of maternal psychological distress on fetal weight, prematurity and intrauterine growth retardation. J. Affect Disord. 111, 214–220.
Malhotra, A., Ditchfield, M., Fahey, M., Castillo-Melendez, M., Allison, B., Polglase, G., Wallace, E., Hodges, R., Jenkin, G., Miller, S., 2017. Detection and assessment of brain injury in the growth restricted fetus and neonate. Pediatr. Res.
Malhotra, A., Ditchfield, M., Fahey, M.C., Castillo-Melendez, M., Allison, B.J., Polglase, G.R., Wallace, E.M., Hodges, R., Jenkin, G., Miller, S.L., 2017. Detection and assessment of brain injury in the growth-restricted fetus and neonate. Pediatr. Res.
Martin, A.J., Friston, K.J., Colebatch, J.G., Frackowiak, R.S.J., 1991. Decreases in regional cerebral blood-flow with Normal aging. J. Cereb. Blood Flow Metab. 11, 684–689.
Martyn, C.N., Barker, D.J.P., Jespersen, S., Greenwald, S., Osmond, C., Berry, C., 1995. Growth in-utero, adult-blood pressure, and arterial compliance. Brit. Heart J. 73, 116–121.
Martyn, C.N., Greenwald, S.E., 1997. Impaired synthesis of elastin in walls of aorta and large conduit arteries during early development as an initiating event in pathogenesis of systemic hypertension. Lancet 350, 953–955.
Mastorci, F., Vicentini, M., Viltart, O., Manghi, M., Graiani, G., Quaini, F., Meerlo, P., Nalivaiko, E., Maccari, S., Sgoifo, A., 2009. Long-term effects of prenatal stress: changes in adult cardiovascular regulation and sensitivity to stress. Neurosci. Biobehav. Rev. 33, 191–203.
Mathewson, K.J., Chow, C.H., Dobson, K.G., Pope, E.I., Schmidt, L.A., Van Lieshout, R.J., 2017. Mental health of extremely low birth weight survivors: a systematic review and meta-analysis. Psychol. Bull. 143, 347.
Matuszek, M.A., Gibson, K.J., Lumbers, E.R., Simonetta, G., 2006. Impact of cortisol on alpha-actin content in vascular smooth muscle cells of fetal sheep. Clin. Exp. Pharmacol. Physiol. 33, 197–203.
McDonald, T.J., Franko, K.L., Brown, J.M., Jenkins, S.L., Nathanielsz, P.W., Nijland, M.J., 2003. Betamethasone in the last week of pregnancy causes fetal growth retardation but not adult hypertension in rats. J. Soc. Gynecol. Invest. 10, 469–473.
McEwen, B., Chao, H., Spencer, R., Brinton, R., MacIsaac, I., Harrelson, A., 1987. Corticosteroid receptors in brain: relationship of receptors to effects in stress and aging. Ann. N.Y. Acad. Sci. 512, 394–401.
McKinlay, C.J.D., Cutfield, W.S., Battin, M.R., Dalziel, S.R., Crowther, C.A., Harding, J.E., Grp, A.S., 2015. Cardiovascular risk factors in children after repeat doses of antenatal glucocorticoids: an RCT. Pediatrics 135, E405–E415.
McMillen, I.C., Robinson, J.S., 2005. Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. Physiol. Rev. 85, 571–633.
Mendelson, T., Dipietro, J.A., Costigan, K.A., Chen, P., Henderson, J.L., 2011. Associations of maternal psychological factors with umbilical and uterine blood flow. J. Psychosom. Obst. Gynecol. 32, 3–9.
Mildenhall, L., Battin, M., Bevan, C., Kuschel, C., Harding, J.E., 2009. Repeat prenatal corticosteroid doses do not alter neonatal blood pressure or myocardial thickness: randomized, controlled trial. Pediatrics 123, E646–E652.
Mildenhall, L.F.J., Battin, M.R., Morton, S.M.B., Bevan, C., Kuschel, C.A., Harding, J.E., 2006. Exposure to repeat doses of antenatal glucocorticoids is associated with altered cardiovascular status after birth. Arch. Dis. Child-Fetal 91, F56–F60.
Millage, A.R., Latuga, M.S., Aschner, J.L., 2016. Effect of perinatal glucocorticoids on vascular health and disease. Pediatr. Res.
Miller, S.L., Huppi, P.S., Mallard, C., 2016. The consequences of fetal growth restriction on brain structure and neurodevelopmental outcome. J. Physiol.-Lond. 594, 807–823.
Miller, S.L., Supramaniam, V.G., Jenkin, G., Walker, D.W., Wallace, E.M., 2009. Cardiovascular responses to maternal betamethasone administration in the intrauterine growth–restricted ovine fetus. Am. J. Obstet. Gynecol. 201 (613), e611–e613, e618.
Miller, S.L., Sutherland, A.E., Supramaniam, V.G., Walker, D.W., Jenkin, G., Wallace, E.M., 2012. Antenatal glucocorticoids reduce growth in appropriately grown and growth-restricted ovine fetuses in a sex-specific manner. Reprod. Fertil. Dev. 24, 753–758.
Moise, A.A., Wearden, M.E., Kozinetz, C.A., Gest, A.L., Welty, S.E., Hansen, T.N., 1995. Antenatal steroids are associated with less need for blood-pressure support in extremely premature-infants. Pediatrics 95, 845–850.
Moisiadis, V.G., Matthews, S.G., 2014. Glucocorticoids and fetal programming part 1: outcomes. Nat. Rev. Endocrinol. 10, 391–402.
Moisiadis, V.G., Matthews, S.G., 2014. Glucocorticoids and fetal programming part 2: mechanisms. Nat. Rev. Endocrinol. 10, 403–411.
Molnar, J., Howe, D.C., Nijland, M.J.M., Nathanielsz, P.W., 2003. Prenatal dexamethasone leads to both endothelial dysfunction and vasodilatory compensation in sheep. J. Physiol.-Lond. 547, 61–66.
Molnar, J., Nijland, M.J.M., Howe, D.C., Nathanielsz, P.W., 2002. Evidence for microvascular dysfunction after prenatal dexamethasone at 0.7, 0.75, and 0.8 gestation in sheep. Am. J. Physiol.-Reg. I 283, R561–R567.
Monk, C., Fifer, W.P., Myers, M.M., Sloan, R.P., Trien, L., Hurtado, A., 2000. Maternal stress responses and anxiety during pregnancy: effects on fetal heart rate. Dev. Psychobiol. 36, 67–77.
Monk, C., Myers, M.M., Sloan, R.P., Ellman, L.M., Fifer, W.P., 2003. Effects of women's stress-elicited physiological activity and chronic anxiety on fetal heart rate. J. Dev. Behav. Pediatr. 24, 32–38.
Monk, C., Newport, D.J., Korotkin, J.H., Long, Q., Knight, B., Stowe, Z.N., 2012. Uterine blood flow in a psychiatric population: impact of maternal depression, anxiety, and psychotropic medication. Biol. Psychiat. 72, 483–490.
Morishima, H.O., Pedersen, H., Finster, M., 1978. The influence of maternal psychological stress on the fetus. Am. J. Obstet. Gynecol. 131, 286–290.

Moritz, K.M., Boon, W.M., Wintour, E.M., 2005. Glucocorticoid programming of adult disease. Cell. Tissue Res. 322, 81–88.
Moritz, K.M., De Matteo, R., Dodic, M., Jefferies, A.J., Arena, D., Wintour, E.M., Probyn, M.E., Bertram, J.F., Singh, R.R., Zanini, S., Evans, R.G., 2011. Prenatal glucocorticoid exposure in the sheep alters renal development in utero: implications for adult renal function and blood pressure control. Am. J. Physiol.-Reg. I 301, R500–R509.
Moritz, K.M., Johnson, K., Douglas-Denton, R., Wintour, E.M., Dodic, M., 2002. Maternal glucocorticoid treatment programs alterations in the renin-angiotensin system of the ovine fetal kidney. Endocrinology 143, 4455–4463.
Moss, T.J.M., Sloboda, D.M., Gurrin, L.C., Harding, R., Challis, J.R.G., Newnham, J.P., 2001. Programming effects in sheep of prenatal growth restriction and glucocorticoid exposure. Am. J. Physiol.-Reg. I 281, R960–R970.
Mulder, E., Derks, J., Zonneveld, M., Bruinse, H., Visser, G., 1994. Transient reduction in fetal activity and heart rate variation after maternal betamethasone administration. Early Hum. Dev. 36, 49–60.
Mulder, E.J., Derks, J.B., Visser, G.H., 2004. Effects of antenatal betamethasone administration on fetal heart rate and behavior in twin pregnancy. Pediatr. Res. 56, 35–39.
Muller, T., Nanan, R., Dietl, J., 2003. Effect of antenatal corticosteroid administration on Doppler flow velocity parameters in pregnancies with absent or reverse end-diastolic flow in the umbilical artery. Acta Obstet. Gyn. Scan. 82, 794–796.
Nagai, M., Hoshide, S., Kario, K., 2010. Hypertension and dementia. Am. J. Hypertens. 23, 116–124.
Nelson, A.R., Sweeney, M.D., Sagare, A.P., Zlokovic, B.V., 2016. Neurovascular dysfunction and neurodegeneration in dementia and Alzheimer's disease. BBA-Mol. Basis Dis. 1862, 887–900.
Nichols, G.G., 1993. Book-ion channels—3. Trends Neurosci. 16, 337.
Nijland, M.J., Ford, S.P., Nathanielsz, P.W., 2008. Prenatal origins of adult disease. Curr. Opin. Obstet. Gyn. 20, 132–138.
Nishina, H., Green, L.R., McGarrigle, H.H.G., Noakes, D.E., Poston, L., Hanson, M.A., 2003. Effect of nutritional restriction in early pregnancy on isolated femoral artery function in mid-gestation fetal sheep. J. Physiol.-Lond. 553, 637–647.
Nuyt, A.M., 2008. Mechanisms underlying developmental programming of elevated blood pressure and vascular dysfunction: evidence from human studies and experimental animal models. Clin. Sci. 114, 1–17.
O'Regan, D., Kenyon, C.J., Seckl, J.R., Holmes, M.C., 2004. Glucocorticoid exposure in late gestation in the rat permanently programs gender-specific differences in adult cardiovascular and metabolic physiology. Am. J. Physiol.-Endoc. Metab. 287, E863–E870.
Okawa, T., Honda, S., Sanpei, M., Ishida, T., Fujimori, K., Sato, A., 2004. Effects of nitric oxide and prostacyclin on hemodynamic response by big endothelin-1 in near term fetal sheep. J. Perinat. Med. 32, 495–499.
Ortiz, L.A., Quan, A., Zarzar, F., Weinberg, A., Baum, M., 2003. Prenatal dexamethasone programs hypertension and renal injury in the rat. Hypertension 41, 328–334.
Packan, D.R., Sapolsky, R.M., 1990. Glucocorticoid endangerment of the hippocampus: tissue, steroid and receptor specificity. Neuroendocrinology 51, 613–618.
Painter, R.C., de Rooij, S.R., Bossuyt, P.M., Phillips, D.I., Osmond, C., Barker, D.J., Bleker, O.P., Roseboom, T.J., 2006. Blood pressure response to psychological stressors in adults after prenatal exposure to the Dutch famine. J. Hypertens. 24, 1771–1778.
Painter, R.C., de Rooij, S.R., Roseboom, T.J., Bossuyt, P.M.M., Simmers, T.A., Osmond, C., Barker, D.J.P., Bleker, O.P., 2005. Early onset of coronary heart disease after prenatal exposure to the Dutch Famine. Pediatr. Res. 58, 1121.
Painter, R.C., Roseboom, T.J., Bleker, O.P., 2005. Prenatal exposure to the Dutch famine and disease in later life: an overview. Reprod. Toxicol. 20, 345–352.
Palinski, W., 2014. Effect of maternal cardiovascular conditions and risk factors on offspring cardiovascular disease. Circulation 129, 2066–2077.
Panel, N.Io.H.C.D., 2001. Antenatal corticosteroids revisited: repeat courses, National Institutes of Health Consensus Development Conference Statement, August 17–18, 2000. Obstetr. Gynecol. 98, 144–150.
Parisi, V.M., Walsh, S.W., 1989. Fetoplacental vascular-responses to prostacyclin after thromboxane-induced vasoconstriction. Am. J. Obstet. Gynecol. 160, 502–507.
Peers, A., Campbell, D.J., Wintour, E.M., Dodic, M., 2001. The peripheral renin-angiotensin system is not involved in the hypertension of sheep exposed to prenatal dexamethasone. Clin. Exp. Pharmacol. Physiol. 28, 306–311.
Phillips, D.I.W., Jones, A., 2006. Fetal programming of autonomic and HPA function: do people who were small babies have enhanced stress responses?. J. Physiol.-Lond. 572, 45–50.
Piazze, J.J., Anceschi, M.M., La Torre, R., Amici, F., Maranghi, L., Cosmi, E.V., 2001. Effect of antenatal betamethasone therapy on maternal-fetal Doppler velocimetry. Early Hum. Dev. 60, 225–232.
Pitale, S., Sahasrabuddhe, A., 2011. Fetal origin of vascular aging. Ind. J. Endocrinol. Metab. 15, S289.
Plana-Ripoll, O., Liu, X.Q., Mornen, N.C., Parner, E., Olsen, J., Li, J., 2016. Prenatal exposure to maternal stress following bereavement and cardiovascular disease: a nationwide population-based and sibling-matched cohort study. Eur. J. Prev. Cardiol. 23, 1018–1028.
Polanska, K., Krol, A., Merecz-Kot, D., Jurewicz, J., Makowiec-Dabrowska, T., Chiarotti, F., Calamandrei, G., Hanke, W., 2017. Maternal stress during pregnancy and neurodevelopmental outcomes of children during the first two years of life. J. Paediatr. Child Health
Pulgar, V.M., Figueroa, J.P., 2006. Antenatal betamethasone administration has a dual effect on adult sheep vascular reactivity. Pediatr. Res. 60, 705–710.
Quaedackers, J.S., Roelfsema, V., Fraser, M., Gunn, A.J., Bennet, L., 2005. Cardiovascular and endocrine effects of a single course of maternal dexamethasone treatment in preterm fetal sheep. BJOG-Int. J. Obstet. Gynaecol. 112, 182–191.
Raaijmakers, A., Zhang, Z.-Y., Claessens, J., Cauwenberghs, N., van Tienoven, T.P., Wei, F.-F., Jacobs, L., Levtchenko, E., Pauwels, S., Kuznetsova, T., 2017. Does extremely Low birth weight predispose to Low-renin hypertension? Novelty and significance. Hypertension 69, 443–449.
Rakers, F., Bischoff, S., Schiffner, R., Haase, M., Rupprecht, S., Kiehntopf, M., Kuhn-Velten, W.N., Schubert, H., Witte, O.W., Nijland, M.J., Nathanielsz, P.W., Schwab, M., 2015. Role of catecholamines in maternal-fetal stress transfer in sheep. Am. J. Obstet. Gynecol. 213.
Rakers, F., Frauendorf, V., Rupprecht, S., Schiffner, R., Bischoff, S.J., Kiehntopf, M., Reinhold, P., Witte, O.W., Schubert, H., Schwab, M., 2013. Effects of early-and late-gestational maternal stress and synthetic glucocorticoid on development of the fetal hypothalamus–pituitary–adrenal axis in sheep. Stress 16, 122–129.
Rakers, F., Rupprecht, S., Dreiling, M., Bergmeier, C., Witte, O.W., Schwab, M., 2017. Transfer of maternal psychosocial stress to the fetus. Neurosci. Biobehav. Rev.
Rakers, F.R., Sven, Dreiling, Michelle, Bergmeier, Christoph, Witte, Otto, W., Schwab, Matthias, 2017. Transfer of maternal psychosocial stress to the fetus. Neurosci. Biobehav. Rev. X, X.
Rasyid, H., Bakri, S., 2016. Intra uterine growth retardation and development of hyperten sion. Acta Med. Indon. 48, 320–324.
Raz, N., Rodrigue, K.M., 2006. Differential aging of the brain: patterns, cognitive correlates and modifiers. Neurosci. Biobehav. Rev. 30, 730–748.
Reul, J., Kloet, Ed., 1985. Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation. Endocrinology 117, 2505–2511.
Robertson, M.C., Murila, F., Tong, S., Baker, L.S., Victor, Y.Y., Wallace, E.M., 2009. Predicting perinatal outcome through changes in umbilical artery Doppler studies after antenatal corticosteroids in the growth-restricted fetus. Obstetr. Gynecol. 113, 636–640.
Roghair, R.D., Lamb, F.S., Bedell, K.A., Smith, O.M., Scholz, T.D., Segar, J.L., 2004. Late-gestation betamethasone enhances coronary artery responsiveness to angiotensin II in fetal sheep. Am. J. Physiol.-Reg. I 286, R80–R88.
Roghair, R.D., Lamb, F.S., Miller, F.J., Scholz, T.D., Segar, J.L., 2005. Early gestation dexamethasone programs enhanced postnatal ovine coronary artery vascular reactivity. Am. J. Physiol.-Reg. I 288, R46–R53.
Roghair, R.D., Segar, J.L., Sharma, R.V., Zimmerman, M.C., Jagadeesha, D.K., Segar, E.M., Scholz, T.D., Lamb, F.S., 2005. Newborn lamb coronary artery reactivity is programmed by early gestation dexamethasone before the onset of systemic hypertension. Am. J. Physiol.-Reg. Integer. 289, R1169–R1176.
Romijn, H.J., Hofman, M.A., Gramsbergen, A., 1991. At what age is the developing cerebral cortex of the rat comparable to that of the full-term newborn human baby?. Early Hum. Dev. 26, 61–67.
Roos, A., Geerts, L., Koen, N., Faure, S.C., Vythilingum, B., Stein, D.J., 2015. Psychosocial predictors of fetoplacental blood flow during pregnancy. Compr. Psychiat. 57, 125–131.
Roseboom, T., de Rooij, S., Painter, R., 2006. The Dutch famine and its long-term consequences for adult health. Early Hum. Dev. 82, 485–491.
Roseboom, T.J., Painter, R.C., van Abeelen, A.F.M., Veenendaal, M.V.E., de Rooij, S.R., 2011. Hungry in the womb: what are the consequences? Lessons from the Dutch famine. Maturitas 70, 141–145.
Rotmensch, S., Liberati, M., Celentano, C., Efrat, Z., Bar-Hava, I., Kovo, M., Golan, A., Moravski, G., Ben-Rafael, Z., 1999. The effect of betamethasone on fetal biophysical activities and Doppler velocimetry of umbilical and middle cerebral arteries. Acta Obstet. Gynecol. Scan. 78, 768–773.
Roussel, S., Hemsworth, P.H., Boissy, A., Duvaux-Ponter, C., 2004. Effects of repeated stress during pregnancy in ewes on the behavioural and physiological responses to stressful events and birth weight of their offspring. Appl. Anim. Behav. Sci. 85, 259–276.
Schütz, S., Le Moullec, J.-M., Corvol, P., Gasc, J.-M., 1996. Early expression of all the components of the renin-angiotensin-system in human development. Am. J. Pathol. 149, 2067.
Schwab, M., Antonow-Schlorke, I., Kühn, B., Müller, T., Schubert, H., Walter, B., Sliwka, U., Nathanielsz, P.W., 2001. Effect of antenatal betamethasone treatment on microtubule-associated proteins MAP1B and MAP2 in fetal sheep. J. Physiol. 530, 497–506.
Schwab, M., Goksaygan, T., Nathanielsz, P.W., 2006. Betamethasone effects on ovine uterine and umbilical placental perfusion at the dose used to enhance fetal lung maturation. Am. J. Obstet. Gynecol. 194, 572–579.
Schwab, M., Roedel, M., Anwar, M.A., Muller, T., Schubert, H., Buchwalder, L.F., Walter, B., Nathanielsz, P.W., 2000. Effects of betamethasone administration to the fetal sheep in late gestation on fetal cerebral blood flow. J. Physiol.-Lond. 528, 619–632.
Seckl, J.R., Meaney, M.J., 2004. Glucocorticoid programming. Ann. N. Y. Acad. Sci. 1032, 63–84.
Segar, J.L., Roghair, R.D., Segar, E.M., Bailey, M.C., Scholz, T.D., Lamb, F.S., 2006. Early gestation dexamethasone alters baroreflex and vascular responses in newborn lambs before hypertension. Am. J. Physiol.-Regual. Integer. 291, R481–R488.
Senat, M.V., Ville, Y., 2000. Effect of steroids on arterial Doppler in intrauterine growth retardation fetuses. Fetal Diagn. Ther. 15, 36–40.
Simeoni, U., Zetterstrom, R., 2005. Long-term circulatory and renal consequences of intrauterine growth restriction. Acta Paediatr. 94, 819–824.
Sjostrom, K., Valentin, L., Thelin, T., Marsal, K., 1997. Maternal anxiety in late pregnancy and fetal hemodynamics. Eur J. Obstet. Gynecol. Rep. Biol. 74, 149–155.
Sjostrom, K., Valentin, L., Thelin, T., Marsal, K., 2002. Maternal anxiety in late pregnancy: effect on fetal movements and fetal heart rate. Early Hum. Dev. 67, 87–100.

Smith, L.M., Ervin, M.G., Wada, N., Ikegami, M., Jobe, A.H., 2003. Single and multiple prenatal glucocorticoid exposures improve preterm newborn lamb cardiovascular and renal function similarly. Am. J. Obstet. Gynecol. 188, 444–453.
Souza, L.V., Oliveira, V., De Meneck, F., Clemente, A.P.G., Strufaldi, M.W.L., do Carmo Franco, M., 2017. Birth weight and its relationship with the cardiac autonomic balance in healthy children.. Plos One 12, e0167328.
Speirs, H.J.L., Seckl, J.R., Brown, R.W., 2004. Ontogeny of glucocorticoid receptor and 11 beta-hydroxysteroid dehydrogenase type-1 gene expression identifies potential critical periods of glucocorticoid susceptibility during development. J. Endocrinol. 181, 105–116.
Tang, L.J., Bi, J.L., Valego, N., Carey, L., Figueroa, J., Chappell, M., Rose, J.C., 2010. Prenatal betamethasone exposure alters renal function in immature sheep: sex differences in effects. Am. J. Physiol-Regual. Integer. 299, R793–R803.
Tangalakis, K., Lumbers, E.R., Moritz, K.M., Towstoless, M.K., Wintour, E.M., 1992. Effect of cortisol on blood-pressure and vascular reactivity in the ovine fetus. Exp. Physiol. 77, 709–717.
Tarjus, A., Amador, C., Michea, L., Jaisser, F., 2015. Vascular mineralocorticoid receptor and blood pressure regulation. Curr. Opin. Pharmacol. 21, 138–144.
Tegethoff, M., Greene, N., Olsen, J., Meyer, A.H., Meinlschmidt, G., 2010. Maternal psychosocial stress during pregnancy and placenta weight: evidence from a national cohort study. Plos One 5.
Teixeira, J.M.A., Fisk, N.M., Glover, V., 1999. Association between maternal anxiety in pregnancy and increased uterine artery resistance index: cohort based study. Brit. Med. J. 318, 153–157.
Thornburg, K.L., 2015. The programming of cardiovascular disease. J. Dev. Orig. Health Dis. 6, 366–376.
Ullian, M.E., 1999. The role of corticosteroids in the regulation of vascular tone. Cardiovasc. Res. 41, 55–64.
UNFPA, W., 2008. Maternal Mental Health and Child Health and Development in Low and Middle Income Countries. Report of UNFPA and WHO, Geneva, Switzerland.
Urban, R., Lernancewicz, A., Przepiesc, J., Urban, J., Kretowska, M., 2005. Antenatal corticosteroid therapy: a comparative study of dexamethasone and betamethasone effects on fetal Doppler flow velocity waveforms. Eur. J. Obstet. Gyn. Rep. Biol. 120, 170–174.
Vaiserman, A.M., 2018. Birth Weight Predicts Aging Trajectory: A Hypothesis. Mechanisms of Ageing and Development.
van den Bergh, B.R.H., 2*, Marion van den Heuvel3,4, Marius Lahti5, Marijke Braeken6, Suzanne R. de Rooij7, Sonja Entringer8,9, Dirk Hoyer10, Tessa Roseboom7,10, Katri Räikkönen5, Suzanne King12, Matthias Schwab13, this issue. Prenatal developmental origins of behavior and mental health problems in humans: the influence of maternal stress in pregnancy. Neurosci Biobehav R xx, xx.
van Dijk, A.E., van Eijsden, M., Stronks, K., Gemke, R.J.B.J., Vrijkotte, T.G.M., 2012. The association between prenatal psychosocial stress and blood pressure in the child at age 5–7 years. Plos One 7.
van Dijk, A.E., van Eijsden, M., Stronks, K., Gemke, R.J.B.J., Vrijkotte, T.G.M., 2014. No associations of prenatal maternal psychosocial stress with fasting glucose metabolism in offspring at 5–6 years of age. J. Dev. Orig. Health Dis. 5, 361–369.
Vythilingum, B., Geerts, L., Fincham, D., Roos, A., Faure, S., Jonkers, J., Stein, D.J., 2010. Association between antenatal distress and uterine artery pulsatility index. Arch. Women Ment. Health 13, 359–364.
Waldstein, S.R., Rice, S.C., Thayer, J.F., Najjar, S.S., Scuteri, A., Zonderman, A.B., 2008. Pulse pressure and pulse wave velocity are related to cognitive decline in the Baltimore longitudinal study of aging. Hypertension 51, 99–104.
Wallace, E.M., Baker, L.S., 1999. Effect of antenatal betamethasone administration on placental vascular resistance. Lancet 353, 1404–1407.
Wang, L., Huang, H., Chen, Y., Cai, H., Qian, J., 2015. The effects of prenatal stress on the cell apoptosis after MCAO in adult offspring ratsZhongguo ying yong sheng li xue za zhi = Zhongguo yingyong shenglixue zazhi = Chinese journal of applied physiology 31, 427–430, 436.
Wang, Y., Fu, W., Liu, J., 2016. Neurodevelopment in children with intrauterine growth restriction: adverse effects and interventions. J. Matern.-Fetal Neo Med. 29, 660–668.
Weinstock, M., 2007. Gender differences in the effects of prenatal stress on brain development and behaviour. Neurochem. Res. 32, 1730–1740.
Weinstock, M., Poltyrev, T., Schorer-Apelbaum, D., Men, D., McCarty, R., 1998. Effect of prenatal stress on plasma corticosterone and catecholamines in response to footshock in rats. Physiol. Behav. 64, 439–444.
Wilkinson, I.B., Cockcroft, J.R., 2004. Commentary: birthweight arterial stiffness and blood pressure: in search of a unifying hypothesis. Int. J. Epidemiol. 33, 161–162.
Wintour, E.M., Johnson, K., Koukoulas, I., Moritz, K., Tersteeg, M., Dodic, M., 2003. Programming the cardiovascular system, kidney and the brain - a review. Placenta 24, S65–S71.
Wyrwoll, C.S., Holmes, M.C., 2012. Prenatal excess glucocorticoid exposure and adult affective disorders: a role for serotonergic and catecholamine pathways. Neuroendocrinology 95, 47–55.
Xiao, D.L., Huang, X.H., Pearce, W.J., Longo, L.D., Zhang, L.B., 2003. Effect of cortisol on norepinephrine-mediated contractions in ovine uterine arteries. Am. J. Physiol.-Heart Circul. 284, H1142–H1151.
Yang, K., Jones, S., Challis, J., 1990. Changes in glucocorticoid receptor number in the hypothalamus and pituitary of the sheep fetus with gestational age and after adrenocorticotropin treatment. Endocrinology 126, 11–17.
Yang, K., Jones, S.A., Challis, J.R.G., 1990. Changes in glucocorticoid receptor number in the hypothalamus and pituitary of the sheep fetus with gestational-age and after adrenocorticotropin treatment. Endocrinology 126, 11–17.
Yang, S., Zhang, L., 2004. Glucocorticoids and vascular reactivity. Curr. Vasc. Pharmacol. 2, 1–12.
Yasue, H., Matsuyama, K., Matsuyama, K., Okumura, K., Morikami, Y., Ogawa, H., 1990. Responses of angiographically Normal human coronary-arteries to intracoronary injection of acetylcholine by age and segment - possible role of early coronary atherosclerosis. Circulation 81, 482–490.
Ylihärsilä, H., Eriksson, J.G., Forsén, T., Laakso, M., Uusitupa, M., Osmond, C., Barker, D.J., 2004. Interactions between peroxisome proliferator-activated receptor-γ2 gene polymorphisms and size at birth on blood pressure and the use of antihypertensive medication. J. Hypertens 22, 1283–1287.

Uncategorized references

Frauendorf, V., Rakers, F., Rupprecht, S., Schubert, H., Schiffner, R., Schwab, M., 2011. Early but not late prenatal stress causes asymmetrical intrauterine growth restriction in fetal sheep. Reprod Sci.. 2455 TELLER RD, THOUSAND OAKS, CA 91320 USA., pp. 269A.
Ogletree, M.L., 1987. Overview of physiological and pathophysiological effects of thromboxane A2. Fed Proc. 133–138.

5 Abschließende Diskussion

Mütterlicher psychischer Stress während der Schwangerschaft, scheint einen wesentlichen Faktor für die Prädisposition von kardiovaskulären und neuropsychischen Erkrankungen im späteren Leben der Nachkommen darzustellen. Epidemiologische Studien zeigen, dass der Fetus eine hohe Plastizität gegenüber pränatalem maternalen Stress besitzt. So kann die kognitive, motorische, physische und verhaltensbasierte Entwicklung der Nachkommen durch maternale psychische Störungen und Naturkatastrophen während der Schwangerschaft negativ beeinflusst werden (King et al. 2012; Van den Bergh et al. 2008). Ein erhöhter Blutdruck bei Kindern pränatal gestresster Mütter ist zudem hinweisgebend, dass MPS negative Aspekte auf die kardiovaskuläre Entwicklung der Nachkommen besitzt (van Dijk et al. 2012, Plana-Ripoll et al., 2016). Die Erforschung der pränatalen Programmierung des erhöhten systemischen Blutdruckes durch MPS als kardiovaskulärer Risikofaktor könnte wegweisend für Neuerungen in der Gesundheitsversorgung sein. Die Wissenschaft beginnt jedoch erst zu verstehen wie psychischer Stress während der Schwangerschaft auf den Fetus übertragen werden kann und sich langfristig auf das kardiovaskuläre System des Fetus auswirkt. Die Programmierung der HPA-Achse und der damit verbundene erhöhte fetale Cortisolspiegel nehmen hierbei eine Schlüsselfunktion in der langfristigen Prägung der Hirnentwicklung (Rakers et al. 2017) und des Gefäßtonus in fetalen renalen und mesenterialen Gefäßen ein. Die Effekte von MPS auf das vaskuläre System, welche zur Prägung der Blutdruckregulation und damit zur Prädisposition kardiovaskulärer Erkrankungen führen können, wurden in dieser Studie am Tiermodell des fetalen Schafes betrachtet. Das fetale Schaf stellt hierfür ein ideales und etabliertes Modell dar, dessen intrauterine Entwicklung viele Parallelen zu der des Menschen aufweist und im Gegensatz zum Menschen, invasive Untersuchungen möglich sind.

Wir konnten zeigen, dass die Reifung der wesentlich an der Blutdruckregulation beteiligten Gefäßsysteme zu unterschiedlichen Zeitpunkten erfolgt (Manuskript 1). Renale Arteriolen erlangen ihre volle Vasoreagibilität und damit Funktion bereits im 2. Trimenon, wohingegen mesenterialen Arteriolen noch im 3. Trimenon reifen.

MPS hat einen Einfluss auf die funktionelle und strukturelle Reifung dieser Gefäße (Manuskript 2). Unabhängig vom Zeitpunkt der Stressung führte MPS zu einer verzögerten Reifung der Gefäßmuskelzellen in mesenterialen aber nicht renalen Gefäßen, was anhand einer veränderten Myosinzusammensetzung deutlich wurde. Die funktionellen Effekte sind heterogen, abhängig vom Gefäßtyp und dem Zeitpunkt der Stressexposition.

Es gibt keine besonders vulnerablen Zeitfenster (1.-2. Trimenon vs. 3. Trimenon) während der fetalen Entwicklung, wie es anzunehmen war. Vielmehr bedingt MPS im 1. bis 2. Trimenon eine Verstärkung der Vasokonstriktion und Dilatation, wobei MPS im letzten Trimenon primär vasodilatative Mechanismen beeinflusst. Dies geht einher mit einer verminderten NO-vermittelten Vasodilatation in renalen und einer verstärkten Vasodilatation in Response zu Acetylcholin in mesenterialen Gefäßen. Der größte Stress-Effekt ist auf Endothelin-1 in mesenterialen Widerstandsgefäßen und auf die NO-vermittelte Vasoreagibilität in renalen Blutgefäßen zurückzuführen. Die MPS-abhängigen Veränderungen der Vasoreagibiltät scheint primär durch die Beeinflussung intrazellulärerer Signaltransduktionswege nicht aber durch Veränderungen der Expression vasomediativer Rezeptoren und Enzym bedingt zu sein. Diese Effekte unmittelbar nach der Stressung sind jedoch 30 Tage danach nicht mehr nachweisbar und wurden durch andere Veränderungen ersetzt. Die verringerte noradrenerge Antwort mesenterialer und verstärkte vasodilatative Antwort renaler Blutgefäße wirkt möglicherweise der initial stressbedingt erhöhten Vasokonstriktion entgegen um diese zu kompensieren.

Unsere Übersichtsarbeit (Manuskript 3) machte deutlich, dass die Effekte von pränataler synthetischer Glukokortikoidexposition auf das kardiovaskuläre System im Tiermodell ebenfalls heterogen sind. Valide Daten zu den Effekten von MPS während der Schwangerschaft im Tiermodell oder dem Menschen liegen kaum vor. Im Vergleich zu unseren Ergebnissen der Auswirkungen von MPS während der Schwangerschaft, können jedoch Parallelen zur Programmierung des vaskulären Systems durch synthetische Glukokortikoide gezogen werden.

5.1 Die Reifung des fetalen mesenterialen (Manuskript 1) und renalen Gefäßsystems (Anhang)

Als Basis für die zu Untersuchung der Effekte von pränatalem Stress auf das vaskuläre System wurde die physiologische Reifung wesentlicher blutdruckregulierender Gefäßsysteme untersucht: des fetalen renalen und mesenterialen Gefäßsystems. Strukturell wurde die physiologische Entwicklung anhand des Verhältnisses von Gefäßwandfläche im Querschnitt zum Lumen (Wall-to lumen-ratio, media cross sectional area) und die Reifung anhand der Expression von fetalem (MHC-B) bzw. adulter (SM2) Myosin-isoform geprüft. Funktionell wurde die Antwort auf verschiedenen Vasomediatoren mittels

small vessel wire myography untersucht und die Expression an der vaskulären Funktion beteiligte Rezeptoren/Enzyme mittels Immunhistochemie geprüft (Tabelle1).

Tabelle 1

Vasomediator	Markertypus	Vasokonstriktion	Vasodilatation
Vasokonstriktoren			
Kalium	-	Depolarisation	
Noradrenalin	Rezeptor	α_{1A}	β_2*
Endothelin-1	Rezeptor	ET_A, ET_B*	ET_B*
Vasodilatatoren			
Acetylcholin	Rezeptor	AChRM2, AChRM3*	
Prostaglandin-E2	Rezeptor		EP2
NO-Donor	Enzym		COX-2*, eNOS*

Tabelle 1. Mittels *small vessel wire myography* untersuchte Vasomediatoren der vaskulären Funktion und anhand von Immunhistochemie untersuchte Rezeptoren und Enzyme, welche wesentlich an der vaskulären Funktion beteiligt sind. Muskarinischer Acetylcholin Rezeptor M2 (AChRM2), muskarinischer Acetylcholin Rezeptor M3 (AChRM3), Adrenorezeptor α1A (α1A), Adrenorezeptor β2 (β2), Cyclooxygenase-2 (COX-2), endotheliale NO Synthase (eNOS), Endothelin-1 Rezeptor Typ A (ETA), Endothelin-1 Rezeptor Typ B (ETB), Prostaglandin-E2-Rezeptor (EP2). * vorwiegend im Endothel exprimiert

Pränatal durchlaufen die renalen Interlobulararteriolen und mesenterialen Widerstandsgefäße eine strukturelle und funktionelle Reifung, zu unterschiedlichen Phasen der Schwangerschaft.

Renale Interlobulararteriolen. Die physiologische Entwicklung der Nieren beginnt bereits im ersten bis mittleren Schwangerschaftsdrittel des Schafes (Wintour and Moritz, 1997). Die konstante Vasokonstriktions- und Vasodilatationskapazität und das Ausbleiben von strukturellen Veränderungen der Gefäßwand von 0.7 bis 0.9 der Gestation unserer Ergebnisse zeigen, dass die funktionelle und strukturelle Reifung der Interlobulararteriolen konkordant zur Nephrogenese, bereits zu Beginn des letzten Trimenons abgeschlossen sind. Dies bildet die Grundlage für die Regulation des fetalen renal-kortikalen Blutflusses als wesentlicher Mediator der Aktivität des RAAS (Wintour and Moritz 1997).

Mesenteriale Arteriolen. Im Gegensatz zu den renalen Gefäßen zeigten die mesenterialen Widerstandsgefäße eine primäre funktionelle Reifung der Vasoreagibilität von 0.7 bis 0.9 der Gestation. Dies bestätigt, dass die Reifung der mesenterialen Gefäße und der renalen

Interlobulargefäße zu unterschiedlichen Phasen der Schwangerschaft erfolgt. Eine strukturelle Reifung scheint jedoch auch bei den mesenterialen Gefäßen bereits zum Zeitpunkt 0.7 der Gestation abgeschlossen. Wir konnten keine Veränderung der Zusammensetzung des fetalen/adulten Myosins oder der „wall-to-lumen-ratio“ in den mesenterialen Gefäßen im letzten Trimenon nachweisen.

Die funktionelle Reifung der mesenterialen Gefäße findet primär im letzten Trimenon statt, was sich in einer gesteigerten Vasokonstriktion sowie einer verstärkten Vasodilatation von 0.7 bis 0.9 der Gestation zeigte. Die Reifung der Endothelfunktion ist geprägt durch eine gesteigerte Vasodilatation in Reaktion auf Acetylcholin und die Reifung der Myozytenfunktion durch eine verstärkte Konstriktion in Response zu Endothelin-1 und Noradrenalin. In Übereinstimmung damit konnte demonstriert werden, dass der vaskuläre Widerstand in Reaktion auf Noradrenalin in mesenterialen Arteriolen von der fetalen Periode bis in die frühe Neonatalperiode hin ansteigt (Lorijn and Longo, 1980, Buckley, 1983). Die gesteigerte Vasoreagibilität in Reaktion auf Endothelin-1 und Noradrenalin wurde auch in femoralen Arteriolen von Schaffeten im letzten Trimenon beschrieben (Docherty et al. 2001), was auf eine simultane funktionelle Entwicklung dieser Widerstandsgefäße hinweist. Die Reifung der mesenterialen Arteriolen ist nicht verbunden mit einer Veränderung der Rezeptor- oder Enzymexpression. Es ist daher zu vermuten, dass die funktionelle Entwicklung im letzten Trimenon eher auf eine Reifung der intrazellulären Signaltransduktionswege zurückzuführen ist. In Übereinstimmung hiermit zeigen andere Studien, das Ca^{2+}- -abhängige und -unabhängige Signalwege der vasomotorischen Kontrolle während der späten Schwangerschaft und frühen Neonatalperiode reifen (Goyal et al. 2008, Goyal et al. 2009, Blood et al. 2002). Reifeprozesse sind für intrazelluläre Ca^{2+}- Speicher, L-type-Ca^{2+}- Kanäle (Blood et al. 2002, Long et al. 2000) und die rho-Kinase-Aktivität (Goyal et al. 2009) in Hirngefäßen, der Karotis und Aorta von fetalen Schafen beschrieben. Die von uns gezeigte Steigerung der mesenterialen Vasokonstriktion und die von anderen gezeigte Steigerung der femoralen Vasokonstriktion (Docherty et al. 2001), trägt höchstwahrscheinlich wesentlich zum Anstieg des peripheren systemischen Widerstands und somit zum fetalen präpartalen Blutdruckanstieg im letzten Trimenon bei (Unno et al. 1999).

Wir können somit Parallelen zwischen den gefäßtypen-spezifischen Unterschieden in der funktionellen Entwicklung von fetalen renalen und mesenterialen Arteriolen und deren physiologischen Funktionen in der fetalen Blutdruckregulation ziehen. Bekannt ist, dass die Nieren durch das Renin-Angiotension-Aldosteron-System (RAAS) an der

mittelfristigen, systemischen Blutdruckregulation beteiligt sind (Dampney et al. 2002). Die mesenterialen Arteriolen, als Vertreter der peripheren Widerstandsgefäße, sind hingegen an der kurzfristigen Blutdruckregulation beteiligt und verengen sich sympathisch vermittelt in Stresssituationen um den Blutdruck zu steigern (Cowley et al. 1992). Die mittelfristige Blutdruckregulation, vermittelt durch das RAAS, an welchem die renalen Interobulararteriolen wesentlich beteiligt sind, scheint somit vor der kurzfristigen Blutdruckregulation durch periphere Widerstandsgefäße zu reifen. Die Regulation des homeostatischen Blutdruckes ist für die Aufrechterhaltung des Blutdruckes im Fetus weit vor der Geburt von großer Bedeutung und scheint daher schon früh in der Entwicklung ausgebildet zu sein, um einen ausreichenden Perfusionsdruck für die zerebrale und viszerale Zikulation zur Verfügung zu stellen. Die Reaktion auf externe Reize wie orthostaische Blutdruckschwankungen und stress-bedingte (sympathisch-vermittelte) Blutumverteilung scheint somit erst mit fortschreitender Entwicklung des Fetus, in Vorbereitung auf die Zeit nach der Geburt, an Bedeutung zu gewinnen.

5.2 Der Einfluss von MPS auf Funktion und Reifung fetaler Gefäßsysteme (Manuskript 2)

Bei der Untersuchung der Auswirkungen von MPS auf die fetale Gefäßreifung müssen die zeitlich versetzte Reifung der Gefäßtypen berücksichtigt werden (siehe Manuskript 1). In beiden Gefäßtypen wurden deshalb die akuten Auswirkungen von MPS im 1. - 2. Trimenon (0.2-0.7 der Gestation) und von MPS im 3. Trimenon (0.7-0.9 der Gestation) untersucht, sowie der prägende Langzeiteffekt von MPS zwischen 0.2-0.7 der Gestation, 30 Tage nach der Stressexposition.

Strukturelle Effekte von MPS

Auf strukturellem Niveau konnten wir in renalen Arteriolen keinen Einfluss von MPS auf die Entwicklung der Gefäßmuskulatur in nachweisen. Im Gegensatz dazu bewirkte MPS, unabhängig vom Zeitpunkt der Stressexposition, eine verzögerte Reifung der glatten Muskelzellen in den mesenterialen Arteriolen, welches sich primär in der verstärkten Expression von fetalem Myosin (MHC-B) gegenüber adultem Myosin (SM2), wiederspiegelte. Die Verzögerung der strukturellen Entwicklung mesenterialer Widerstandsgefäße impliziert, dass dieser Gefäßtyp vulnerabler gegenüber MPS ist als es renale Interlobulararteriolen sind. Im Gegensatz zu unserer Hypothese der besonderen Stressvulnerabili-

tät mesenterialer Gefäße im. 3. Trimenons traten die Stresseffekte sowohl durch Exposition im 1.-2. Sowie im 3. Trimenon auf, d.h. auch nach Abschluss der strukturellen Gefäßreifung am Ende des 2. Trimenons (siehe 5.1 Die Reifung des fetalen mesenterialen (Manuskript 1) und renalen Gefäßsystems (Anhang)). Diese strukturellen Stresseffekte konnten auch 30 Tage nach Ende der Stressexposition festgestellt werden, was für einen prägenden Effekt der Stressexposition auf mesenteriale, nicht jedoch auf renale Gefäße, spricht.

Funktionelle Effekte von MPS

Auf funktioneller Ebene konnten sowohl im renalen als auch mesenterialen Blutgefäßen stress-induzierte Veränderungen nachgewiesen werden. Die Effekte waren bezüglich des Zeitpunktes der Stressexposition als auch zwischen den beiden Gefäßtypen heterogen und betrafen vasokonstriktorische und vasodilatative Signalwege. Damit standen die Effekte im Gegensatz zu unserer Hypothese der besonderen Stressvulnerabilität der Widerstandsgefäße zum Zeitpunkt der primären Reifung der Gefäßsysteme (siehe 5.1). Im Detail hatte MPS im 1.- 2. und im 3. Trimenon folgende Effekte.

1.-2. Trimenon (0.2-0.7 der Gestation)

MPS zwischen 0.2-0.7 der Gestation bewirkte nach Ende der Stressexposition eine verstärkte Endothelin-1 vermittelte Vasokonstriktion sowohl in renalen als mesenterialen Gefäßen. In den renalen Gefäßen war zusätzlich eine verstärkte endotheliale Vasodilatation in Reaktion auf Acetylcholin nachweisbar.

Die verstärkte Vasoreagibiltät auf Endothelin-1 und Acetylcholin war zum Zeitpunkt 0.9 der Gestation (d.h. 30 Tage nach Ende der Stressexposition) nicht mehr nachweisbar. Zu diesem Zeitpunkt waren vasodilatative Komponenten verstärkt, möglicherweise um die initial stressbedingt erhöhte Reaktion auf die Endothelin-1-abhängige Vasokonstriktion zu kompensieren. Allerdings unterschieden sich die Langzeiteffekte zwischen den untersuchten Gefäßtypen. Sie umfassten eine verstärkte Progstaglandin-E2 (PGE2) und NO-abhängige Vasodilatation im renalen Blutgefäß und eine geringere Gefäßgrundspannung sowie eine verringerte noradrenerge Vasokonstriktion im mesenterialen Blutgefäß. Zusammengefasst bestätigt dies unsere zweite Hypothese. Durch MPS werden Glukokortikoid-sensitive Vasotransmittersysteme beeinflusst (Endothelin-1, Acetylcholine, NO, Prostaglandine), im Speziellen die funktionelle Reifung des Endothels. Die funktionellen Adaptionen an MPS waren jedoch nicht von einer veränderten Rezeptor- (AChRM2, AChRM2, EP2, ET_A, ET_B, α_{1A}, β_2) bzw. Enzymexpression (eNOS, COX-2) begleitet

Insgesamt kompensierte 30 Tage nach Ende der Stressexposition im 1. und 2. Trimenon die Verschiebung zu einer stärkeren Vasodilatation in beiden Gefäßsystemen die kurz nach Stressexposition nachweisbar erhöhte Vasokonstriktion. Die Programmierung eines erhöhten Blutdruckes, wie er in epidemiologischen Studien beschrieben wurde (van Dijk et al. 2012, Plana-Ripoll et al., 2016) scheint demnach bisher nicht auf vaskuläre Stresseffekte zurückgeführt werden können. Die Parallelen zu den Effekten nach pränataler Exposition zu synthetischen Glukokortikoiden wird im nächsten Kapitel diskutiert.

3. Trimenon (0.7-0.9 der Gestation)

Analog zu den Effekten von MPS im 1.-2. Trimenon verstärkt auch MPS im letzten Trimenon (0.7-0.9 der Gestation) primär vasodilatative Komponenten der Vasoreagbilität in beiden Gefäßtypen, wobei sich die durch MPS im letzten Trimenon beeinflussten Vasomediatoren von den durch MPS im 1.-2. Trimenon beeinflussten Vasomediatoren unterscheiden. Durch MPS im letzten Trimenon wurde die endothelunabhängige und –abhängige NO und PGE2-vermittelte Vasodilatation in renalen Gefäßen vermindert, die endothelabhängige Vasodilatation (Acetylcholin) in mesenterialen Gefäßen verstärkt.

Analog zu MPS im 1.-2. Trimenon war die funktionell veränderte Vasoreagibilität nicht von Änderungen einer korrespondierenden Rezeptor- (AChRM2, AChRM2, EP2, ET_A, ET_B, α_{1A}, β_2) bzw. Enzymexpression (eNOS, COX-2) begleitet. Entgegen unserer dritten Hypothese hat MPS im letzten Trimenon, trotz der scheinbar bereits abgeschlossenen physiologischen Reifung der renalen Interlobulararteriolen (siehe Anhang) auch Auswirkung auf die Entwicklung dieses Gefäßsystems (Manuskript 2).

Insgesamt führt MPS im letzten Trimenon zwar in beiden Gefäßsystemen zu einer Störung der vasodilatativen Kapazität, welche jedoch nicht den erhöhten systemischen Blutdruck von Kindern von Müttern nach psychischem Stress während der Schwangerschaft erklärt (van Dijk et al. 2012, Plana-Ripoll et al., 2016). Der Vergleich zu Studien nach synthetischer Glukokortikoidexposition wird im nächsten Kapitel gezogen.

Die entwicklungsbezogene Plastizität von Blutgefäßen *in utero* gegenüber äußeren Einflüssen (z.B. synthetischen Glukokortikoiden etc.) ist schon seit langem bekannt (Seckl, 2001). Exogene Glukokortikoide können prägende blutdrucksteigernde Effekte auf das Kreislaufsystem des Ungeborenen haben (Seckl, 2001). Durch unsere Studie wird nun deutlich, dass auch MPS während kritischer Phasen der fetalen Entwicklung die Struktur und Funktion wesentlicher an der Blutdruckregulation beteiligter Gefäßsysteme beein-

flusst. Wir konnten allerdings keine Stressperiode (1.-2. Trimenon, oder letztes Trimenon) identifizieren, für welche die Gefäßtypen vulnerabler sind. In beiden Zeiträumen wurde die Vasoreagibilität über unterschiedliche Mediatoren durch MPS beeinflusst. Darüber hinaus hatte MPS, abhängig vom untersuchten Gefäßtyp unterschiedliche Einflüsse auf die Gefäßreagibilität. Diese Unterschiede sind durch unsere Untersuchungen nicht zu erklären.

5.3 Die Auswirkungen von MPS auf die fetale Gefäßentwicklung - Vergleich zu Studien mit synthetischen Glukokortikoiden (Manuskript 3)

Wesentlicher Mediator von MPS ist Cortisol (Dodic et al. 1998), was den Vergleich unsere Ergebnisse zu den Effekten der systemischen Administration von synthetischen Glukokortikoiden nahelegt. Die meisten bisherigen Untersuchungen zu den Auswirkungen von pränatalem Stress wurden nach Applikation von synthetischen Glukokortikoiden im Schaf durchgeführt (Bsp.: 1. Trimenon: Roghair et al. 2005, 2. Trimenon: Dodic et al. 1998, Hai et al. 2002, 3. Trimenon: Molnar et al. 2003, Roghair et al. 2004). Im Folgenden sollen nun die Effekte von MPS während der Schwangerschaft mit der pränatalen Exposition zu synthetischen Glukokortikoiden verglichen werden.

Strukturelle Gefäßveränderungen

Pränatal applizierte synthetische Glukokortikoide und MPS verändern den strukturellen fetalen Gefäßaufbau (McMillen & Robinson, 2005, Hai et al. 2002). Dabei gibt es Unterschiede. Synthetische Glukokortkoide führen zu einer mikroskopischen Änderung im Gefäßaufbau (McMillen & Robinson, 2005). In unserer Studie hingegen gab es, unabhängig vom Zeitpunkt der Stressexposition keinen Anhalt für Hypertrophie oder eine gesteigerte Proliferation der Gefäßmuskelzellen durch MPS, was ein altersgerechter Durchmesser der Gefäßmuskelschicht (*media cross-sectional area*, *media-to-lumen-ratio*) beweist. Ein Weiterer Fokus der Untersuchungen zur Gefäßstruktur in unserer Studie lag auf der fetalen und adulten Myosin Expression. Nach pränataler Exposition zu synthetischen Glukokortikoiden ist eine Veränderung der fetalen/adulten Myosin-Zusammensetzung beschrieben (Hai et al. 2002). Analog zu diesen Ergebnissen konnten wir eine verstärkte Expression des fetalen Myosins (MHC-B) beobachten, welches hinweisgebend für die durch MPS verzögerte strukturelle vaskuläre Entwicklung ist. Weitere strukturelle Veränderungen nach synthetischer Glukokortikoidexposition umfassen einen verminderten

Elastin- und erhöhten α-Aktin-Gehalt in der Gefäßwand als Marker einer gesteigerten Gefäßrigidität (Matuszek et al. 2006, Martyn et al. 1995, Nuyt 2008). In wie weit MPS einen Einfluss auf die Gefäßelastizität bzw. auf die Elastin- und α-Aktin Expression hat, wurde in unserer Studie nicht untersucht. Die mikroskopisch erkennbaren Effekte nach synthetischen Glukokortikoidexposition wie wir sie nach MPS nicht beobachten konnten, sind möglicherweise auf die stärkere Bioaktivität und den ungehemmten Plazentaübertritt der synthetischen Glukokortikoide zurückzuführen.

Funktionelle Gefäßveränderungen

Die funktionellen Effekte von MPS sind vielseitig und einige Parallelen können zu Effekten nach Applikation von synthetischen Glukokortikoiden im gleichen Tiermodell gezogen werden. In femoralen Widerstandsgefäßen (Docherty et al. 2001; Molnar et al. 2003; Molnar et al. 2002), wie auch durch uns ermittelt in mesenterialen und renalen Arteriolen (Manuskript 2), führten synthetische, bzw. endogene Glukokortikoide zu einer gesteigerten Endothelin-1-abhängigen Vasokonstriktion. Auch die Reaktion zur endothelabhängigen Vasodilatation zu Acetylcholin war vergleichbar. So zeigten Femoralarteriolen und Mesenterialarteriolen von pränatal gestressten (Manuskript 2) bzw. mit synthetischen Glukokortikoid-behandelten Feten (Anwar et al., 1999; Roghair et al., 2004; Manuskript 2) eine höhere Sensitivität zu Acetylcholin. Interessant ist, dass der Effekt nach Applikation von synthetischen Glukokortikoiden im letzten Trimenon auf die Acetylcholin-Sensitivität scheinbar bis in das Erwachsenenalter reicht (Molnar et al. 2003; Pulgar & Figueroa, 2006; Roghair et al. 2005). Die Auswirkungen von synthetischen Glukokortikoiden auf andere Vasokonstriktoren (K^+, Phenylephrin, Norepinephrin, Thromboxan-A2, Angiotensin-II) und Vasodilatatoren (Sodium Nitroprusside) wurde in femoralen und mesenterialen Widerstandsgefäßen untersucht (Roghair et al. 2004, Roghair et al. 2005, Segar et al. 2006, Anwar et al. 1999, Docherty et al. 2001, Molar et al. 2002, Molar et al. 2003). Die Ergebnisse der Untersuchungen zur Beeinflussung des Vasotonus durch diese Vasomediatoren sind jedoch zu divers um die Rolle des Expositionszeitpunktes, der Expositionsdauer und dem Zeitraum zwischen Exposition und Untersuchungszeitpunkt zu identifizieren (Manuskript 3).

Die Effekte von MPS im 1.-2. Trimenon in mesenterialen und renalen Gefäßen waren 30 Tage nach Ende der Stressexposition nicht mehr nachweisbar (Manuskript 2) und wurde durch andere Effekte ersetzt. Wie für synthetische Glukokortikoide gezeigt (Moritz et al.

2005), können damit Veränderungen der Vasoreagibilität auch verzögert im juvenilen oder adulten Nachwuchs auftreten. Die funktionelle Untersuchung der prägenden vaskulären Effekte von MPS im juvenilen oder adulten Nachwuchs stellt somit ein interessantes, zukünftiges Forschungsfeld dar.

Die Parallelen lassen sich durch die gemeinsame Wirkung von Cortisol und synthetischen Glukokortikoiden am Glukokortikoidrezeptor erklären, welcher als Transkriptionsfaktor multiple Prozesse nachhaltig beeinflusst und schon früh im fetalem Gewebe exprimiert wird (Yang et al. 1990).

Es gibt jedoch auch unterschiedliche Effekte von MPS und der Exposition zu synthetischen Glukokortikoiden auf die vaskuläre Entwicklung. Die Unterschiede zwischen MPS und der Exposition zu synthetischen Glukokortikoiden wird beim Vergleich der endothelunabhängigen, NO-bedingten Vasodilatation deutlich. In mesenterialen Arteriolen hatte MPS keinen Effekt auf die NO-abhängige Dilatation, wohingegen die pränatale Exposition zu synthetischen Glukokortikoiden eine verminderte NO-abhängige Vasodilatation zur Folge hatte (Roghair et al. 2004). Durch MPS und synthetische Glukokortikoide werden möglicherweise Vasomediatorensysteme angesprochen, welche im Speziellen die funktionelle Reifung des Endothels (Endothelin-1 - und Acetylcholinantwort) beschleunigen. Die zusätzlichen Effekte von MPS (NO-Antwort) auf die Widerstandsgefäße kann vielfältige Ursachen haben. So führt MPS nicht nur zur Freisetzung von Cortisol, sondern im Gegensatz zu synthetischen Glukokortikoiden, auch zur Freisetzung von Katecholaminen, welche selbst eine vasokonstriktive Wirkung haben (Derks et al., 1997; Rakers et al. 2015). Während letztere zwar nicht plazentagängig sind (Jones and Robinson 1975), so führt doch die Aktivierung des mütterlichen sympathischen Nervensystems durch MPS zu einem erhöhten vaskulären Tonus in Plazenta und im Uterus. Damit einher geht eine verringerte plazentare Durchblutung, die ebenfalls einen fetalen Stressor darstellt (Rakers et al. 2017). Unabhängig vom Plazentatransfer von mütterlichem Cortisol kommt es zu einer fetalen Produktion von Katecholaminen während des pränatalen Stressinsults (Rakers et al. 2015). Katecholamine als potente Vasokonstriktoren, tragen mit hoher Wahrscheinlichkeit zu den beobachteten vaskulären Effekten von MPS bei.

Signaltransduktionswege

Entgegen den Effekten nach Therapie mit synthetischen Glukokortikoiden (AT1-Rezeptor: z.B. Roghair et al. 2003, ETA: Kutzler et al. 2002), führten unsere ermittelten Stress-

bedingten funktionellen Veränderungen nicht zu eindeutigen Veränderung der korrespondierenden Enzym- und Rezeptorexpression in den untersuchten fetalen Gefäßsystemen. Wir schließen daraus, dass die funktionellen, stressbedingten Änderungen der Vasoreagibilität nicht auf Unterschiede in der Rezeptorendichte oder -Affinität, sondern wahrscheinlich auf eine Modifikation intrazellulärer Signalkaskaden zurückzuführen sind. Vielmehr scheint eine pränatale Exposition zu synthetischen Glukokortikoiden eine Vielzahl intrazellulärer Prozesse, welche auf die Kontraktilität der vaskulären Muskelzelle Auswirkungen hat, nachhaltig zu prägen (Lewko et al. 1993, Lee et al. 2014, Slotkin et al. 1994). Gemeinsamkeiten der Effekte von synthetischen Glukokortikoiden und MPS auf die Vasokontraktions- (Endotehlin-1) und Vasodilatationsmechanismen (Acetylcholin) des Fetus, lassen sich durch die gemeinsame Wirkung von Cortisol und synthetischen Glukokortikoiden am Glukokortikoidrezeptor erklären, welcher als Transkriptionsfaktor multiple Prozesse nachhaltig beeinflusst und schon früh im fetalem Gewebe exprimiert wird (Yang et al. 1990).

Beispiele für einen durch synthetische Glukokortikoide beeinflussten Signaltransduktionsweg ist die Expression der Adenylat Zyklase (Abbildung 2), welche durch synthetische Glukokortikoide hochreguliert wird (Slotkin et al. 1994; Bian et al. 1992; Stein et al. 1993). Dieses Enzym ist durch die Produktion von cAMP an multiplen Signalwegen, unter anderem der Vasoreagibilität in vaskulären Muskelzellen beteiligt (Abbildung 2). Die Aktivierung des Enzyms führt zur Amplifizierung des initialen Signals und realisiert so unter anderem die Membranrezeptor-abhängige Vasodilatation (Abbildung 2). Weitere durch Glukokortikoide modulierte Signalwege sind die Ca^{2+} Mobilisierung (Lee et al.2014, Wenjie et al. 2014) und die Aktivität der löslichen Guanylatzyklase (Lewko et al. 1993).

Die Unterschiede der Effekte von synthetischen Glukokortikoiden und MPS liegt möglicherweise in dem weitaus komplexeren Wirkmechanismus von MPS und der höheren biologischen Potenz synthetischer Glukokortikoide begründet.

Die funktionelle Untersuchung der vaskulären Effekte und der korrespondierenden Signalwege nach pränataler Stressexposition im juvenilen und adulten Nachwuchs stellt somit ein interessantes, zukünftiges Forschungsfeld dar.

5.4 Kardiovaskuläre Effekte von MPS und synthetischen Glukokortikoiden im späteren Leben (Manuskript 3)

In der Übersichtsarbeit haben wir den aktuellen Wissensstand zu den prägenden Effekten von MPS auf das kardiovaskuläre System zusammengefasst und den Effekten von synthetischen Glukokortikoiden gegenüberstellt. Dabei wurde die Literatur zu den Auswirkungen von maternalem psychischen Stress bzw. MPS auf das kardiovaskuläre System des Fetus/Nachwuchs aus epidemiologischen und experimentellen Studien gegenübergestellt und mit Daten zur pränatalen Exposition zu synthetischen Glukokortikoiden verglichen.

Die Untersuchungen zur peripheren fetalen Durchblutung haben zum Großteil in Tiermodellen unter Verwendung von synthetischen Glukokortikoiden stattgefunden. Akut verursachte eine pränatale Glukokortikoidexposition im letzten Trimenon reproduzierbar einen transienten Blutdruckanstieg (Quaedacker et al. 2005; Docherty et al. 2001; Derks et a. 1997), welcher jedoch nicht in die neonatale/juvenile Phase anhielt (Moss et al. 2001). Die Einwirkung von synthetischen Glukokortikoiden im 1. Trimenon verursachte einen Blutdruckanstieg, welcher nicht im neonatalen (Segar et al. 2006, Moritz et al. 2002), wohl aber im juvenilen und adulten Tier beobachtet werden konnte (Roghair et al. 2005; Dodic et al. 1998; Peers et al. 2001). Somit ist eine vulnerable Phase für die Programmierung des fetalen Blutdruckes durch exogene Glukokortikoide vor allem während der frühen Schwangerschaft (0.2 der Gestation) belegt. Eine Rolle für diesen prägenden Effekt auf die Blutdruckregulation scheinen synthetische Gukokortikoide durch die Prägung der Herz-und Nierenentwicklung (Moritz et al. 2002) und die Modulation des RAAS (Moritz et al. 2002) sowie der HPA-Achse (Wintour & Moritz et al. 1997) zu spielen.

Ähnlich wie nach synthetischer Glukokortikoidexposition konnte in unserer Arbeitsgruppe nach MPS im letzten Trimenon ein transienter Blutdruckanstieg des Fetus nachgewiesen werden (Dreiling et al. 2017; Rakers et al. 2015). Tierexperimentelle Studien zu den prägenden Auswirkungen von MPS auf das kardiovaskuläre System sind rar und konnten keinen basalen Blutdruckanstieg im juvenilen/adulten Nachwuchs von gestressten Ratten und Meerschweinchen (Ratte: Igosheva et al. 2004; Meerschweinchen: Kapoor and Matthews 2005) nachweisen, aber eine höhere mittlere Herzfrequenz bei juvenilen pränatal gestressten Schafen während induzierter Stressphasen (Roussel et al. 2004). Die länger anhaltenden Effekte synthetischer Glukokortikoide im Vergleich zu MPS sind vermutlich zurückzuführen auf die weitgehende Inaktivierung des maternalen Cortisols

durch die plazentare 11β-HSD2 (Seckl & Meaney, 2004) und die höhere biologische Potenz synthetischer Glukokortikoide (Yang et al. 1990).

Humanen Studien mangelt es bezüglich der akuten Effekte einer pränatalen Applikation von synthetischen Glukokortikoiden aufgrund des heterogener Studiendesigns und einer geringen Fallzahl an Aussagekraft (Malhorta et a. 2017). Bisher gibt es keine humane Langzeitstudie, welche einen Zusammenhang zwischen pränataler Glukokortikoidexposition und einem negativen Effekt auf die kardiovaskuläre Gesundheit herstellt. Wenige prospektive Studien konnten jedoch einen schwachen Zusammenhang zwischen maternalem psychischen Stress und dem erhöhten Blutdruck des Nachwuchses in der Kindheit und im Erwachsenenalter (van Dijk et al. 2012; Plana-Ripoll et al., 2016) beobachten. Umfangreiche prospektive Studien nach pränataler Glukokortikoid Applikation sowie MPS beim Menschen wären demnach von Nöten, um die kardiovaskulären Auswirkungen des Nachwuchses in Stresssituationen zu untersuchen und um gezieltere tierexperimentelle Studien zu den Mechanismen anschließen zu können. Allerdings sind diese aufgrund ihrer diffizilen Durchführung limitiert.

5.5 Limitationen der Methoden und Ergebnisse

In dieser Arbeit wurde die Reifung der fetalen mesenterialen Widerstandsgefäßen und renalen Interlobulararteriolen anhand struktureller und funktioneller Methoden im Tiermodell des fetalen Schafes charakterisiert. Mechanistische humane Studien sind aus ethischen Gründen nicht durchführbar, da sich eine quantifizierbare Exposition der werdenden Mütter gegenüber experimentellen Stressoren verbietet. Zudem sind prospektive humane Studien aufgrund des notwendigen langen Beobachtunsgzeitraums nur schwer durchführbar. Das fetale Schaf hat sich, als klassisches Tiermodell für Untersuchungen der humanen Fetalphysiologie etabliert, da der zeitliche Ablauf der intrauterinen Entwicklung dem des menschlichen Feten ähnelt, im Gegensatz zu kleineren Versuchstieren wie Nagern, die extrem unreif zur Welt kommen (Andersen et al. 2018). Die Größe der Feten und der ähnliche Ablauf der intrauterinen Entwicklung der Feten von Schaf und Mensch lässt ein Studium und den Vergleich der wesentlichen in der Blutdruckregulation involvierten Organsysteme zu. Unsere Arbeitsgruppe etablierte auf dieser Grundlage ein Tiermodell an Schafen mit reproduzierbar erhöhten maternalen Cortisolspiegeln, welche durch chronischen psychosozialen Stress während der Schwan-

gerschaft induziert wurden. Das chronisch gestresste fetale Schaf bietet somit ein adäquates Tiermodell, um die Effekte und die Mechanismen der Stressübertragung von der Mutter auf den Fetus zu charakterisieren und potentiell die Programmierung von Krankheiten im späteren Leben zu untersuchen.

Die funktionellen Untersuchungen der fetalen Blutgefäße wurden mittels *small vessel wire myography* durchgeführt. Eine Methode, bei der sich die Funktion vitaler Gefäße mit einem Durchmesser von ca. 200µm unter Einwirkung verschiedener Vasomediatoren *ex vivo* reproduzierbar untersuchen lässt (Mulvany et al. 1977). Diese Methode erlaubt die funktionelle Untersuchung von Widerstandsgefäßen sowie zerebrovaskulären Gefäßen im Großtiermodell und von Konduitgefäßen bei Nagern auf mechanischer Ebene (Mulvany & Aalkjær 1990). Die Widerstandsgefäße bei Nagern sind zu kleinlumig für die funktionelle Untersuchung und Konduitgefäße spiegeln nicht das Verhalten blutdruckregulierender Arteriolen wider, daher stellte ein Großtiermodell wie das fetale Schaf die adäquate Wahl für unsere Untersuchungsziele dar.

Die strukturellen Untersuchungen der Gefäßtypen erfolgte an Gefäßquerschnitten, welche die Untersuchung der mikroskopischen Gefäßstrukturen zuließ. Mittels Immunhistochemie konnte weiterhin die Lokalisierung der Expression vasoaktiver Rezeptoren und Enzyme semiquantitativ dargestellt werden. Nachteilig an dieser Methode ist der Verlust an Quantifizierbarkeit gegenüber dem Western-Blot-Verfahren, bei welchem wiederum eine räumliche Auflösung der Rezeptor-und Enzymexpression nicht möglich gewesen wäre. Das genutzte immuhistochemische Verfahren ermöglichte die genaue Lokalisierung der untersuchten Proteine und deren semiquantitative Expressionsanalyse und bot somit die adäquate Untersuchungsmethode für unsere Belange.

Wie sich MPS auf die kardiovaskuläre Entwicklung des Fetus in den ersten Wochen der Schwangerschaft auswirkt, kann experimentell kaum untersucht werden, da eine Stressexposition kurz nach der Konzeption in vielen Fällen zum Abort führt (Catanzaro & Macniven 1991). Von Studien nach pränataler Applikation von synthetischen Glukokortikoiden im letzten Trimenon wissen wir, dass der erhöhte Blutdruck häufig erst im juvenilen bzw. adulten Nachkommen nachweisbar ist (Dodic et al. 1998, Peers et al. 2001, Roghair et al. 2005, Wintour et al. 2003). Über die Studien nach pränataler Applikation von synthetischen Glukokortikoiden (Manuskript 3) hinausgehend, ist im Tiermodell nichts bekannt über die prägenden vaskulären Effekte von MPS im späteren Leben der Nachkommen.

6 Schlussfolgerung & Ausblick

In dieser Arbeit wurde die Reifung der fetalen mesenterialen Widerstandsgefäßen und renalen Interlobulararteriolen und die Modulation der Reifung durch MPS anhand struktureller und funktioneller Methoden charakterisiert. Die Ergebnisse geben einen Einblick in die Entwicklung der fetalen Vasoreagibilität und den Einfluss von MPS auf diese. Wir konnten multiple und auch langfristige Effekte von MPS auf die funktionelle und strukturelle Reifung von fetalen Gefäßsystemen, welche in die Blutdruckregulation eingebunden sind, zeigen. Weiterhin konnte ein gefäßtypenspezifischer Effekt beschrieben werden, der abhängig vom Zeitpunkt der Stressexposition ist und Auswirkungen auf die kurz- und mittelfristige Blutdruckregulation des Fetus hat. In vorhergehenden tierexperimentellen Studien konnten mittels maternaler Applikation von synthetischen Glukokortikoiden zum Teil ähnliche Effekte festgestellt werden, jedoch wurden dabei andere vulnerable Phasen während der Schwangerschaft im Vergleich zur Induktion von MPS beobachtet. Inwieweit diese Effekte zu einer Prädisposition für kardiovaskuläre Erkrankungen beitragen, müssen Untersuchungen der Vasoreagibilität an adulten Nachkommen zeigen. Dabei sollte beachtet werden, dass MPS mit hoher Wahrscheinlichkeit intrazelluläre Signaltransduktionswege beeinflusst, welche für die untersuchten Vasokonstriktions- und -dilatationsmechanismen essentiell sind.

Rückschlüsse auf die zugrunde liegenden Mechanismen beim Menschen sind aus tierexperimentellen Studien nicht in vollem Umfang möglich. Allerdings wären gesicherte Erkenntnisse hierzu bedeutend, da psychischer Stress, ein Symptom unserer leistungsorientierten Gesellschaft, besonders in der Geburtsmedizin ein wesentlicher Einflussfaktor ist. Circa 10-15% aller Frauen in Hochlohnländern (Falah-Hassani et a. 2017) berichten von psychischem Stress während der Schwangerschaft. Der prägende Effekt von maternalem psychischem Stress auf fetale blutdruckregulierende Blutgefäße, und damit auf die kardiovaskuläre Gesundheit der nächsten Generation, ist nicht belegt.

Daher ist es wichtig, dass in weiteren experimentellen und klinischen Studien die Konsequenzen einer durch pränatalen Stress veränderten Vasoreagibilität im späteren Leben untersucht werden, um den Aspekt der vaskulären der Stressvulnerabilität und Resilienz näher zu erforschen. Nur wenige prospektive Studien konnten bisher einen schwachen Zusammenhang zwischen maternalem psychischen Stress und erhöhtem Blutdruck der Nachkommen in Kindheit und Erwachsenenalter herstellen (van Dijk et al. 2012; Plana-Ripoll et al., 2016). Neben Studien an Gefäßsystemen, welche in die Blutdruckregulation

involviert sind, zum Beispiel dem renalen und dem mesenterialen Gefäßsystem, ist die Untersuchung der programmierenden Effekte von MPS auch in anderen Gefäßbetten, wie dem zerebrovaskulären System, wichtig.

Im Nagermodell konnte gezeigt werden, dass pränataler Stress mit einer geringeren neuronalen Plastizität nach einem Schlaganfall assoziiert ist (Faraji J et al. 2017). Diese Verringerung der Plastizität ist möglicherweise auf eine verminderte reaktive Vasodilatation in der Penumbra nach Schlaganfall zurückzuführen. Diese Erkenntnisse verdeutlichen die Bandbreite möglicher Einflüsse und werfen weitere Fragen bezüglich der Auswirkungen von MPS auf die kardio- und zerebrovaskuläre Gesundheit auf. Demgegenüber müsste diskutiert werden ob MPS auch positive Auswirkungen besitzt, welche Stresslevel pathologisch sind und ob diese objektiv ermittelt werden können. Zudem sollte der Aspekt der geschlechtsspezifischen Unterschiede bei den Auswirkungen auf den Nachwuchs untersucht werden.

Um in Zukunft Maßnahmen und Empfehlungen zur Prävention der weltweit häufigsten Todesursache – den kardiovaskulären Erkrankungen – entwickeln und ableiten zu können, müssen die Hintergründe und Mechanismen der fetalen Programmierung beim Menschen weiter erforscht werden.“

Literaturverzeichnis

1. Alexander BT, Dasinger JH, and Intapad S. Fetal programming and cardiovascular pathology. Comprehensive Physiology 5: 997-1025, 2011.

2. Andersen MD, Alstrup AKO, Duvald CS, Mikkelsen EFR, Vendelbo MH, Ovesen PG, and Pedersen M. Animal Models of Fetal Medicine and Obstetrics. Experimental Animal Models of Human Diseases: An Effective Therapeutic Strategy: 343, 2018.

3. Anwar MA, Docherty C, Poston L, and Nathanielsz PW. A comparative study of vascular responsiveness of myometrial and omental small resistance arteries in late-gestation sheep. Am J Obstet Gynecol 181: 663-668, 1999.

4. Banjanin S, Kapoor A, and Matthews SG. Prenatal glucocorticoid exposure alters hypothalamic–pituitary–adrenal function and blood pressure in mature male guinea pigs. The Journal of physiology 558: 305-318, 2004.

5. Barker D, Osmond C, Simmonds S, and Wield G. The relation of small head circumference and thinness at birth to death from cardiovascular disease in adult life. Bmj 306: 422-426, 1993.

6. Barker DJ. The fetal and infant origins of adult disease. BMJ: British Medical Journal 301: 1111, 1990.

7. Barker DJ. Fetal nutrition and cardiovascular disease in later life. Brit Med Bull 53: 96-108, 1997.

8. Bateson P, Barker D, Clutton-Brock T, Deb D, D'Udine B, Foley RA, Gluckman P, Godfrey K, Kirkwood T, and Lahr MM. Developmental plasticity and human health. Nature 430: 419, 2004.

9. Beijers R, Buitelaar JK, and de Weerth C. Mechanisms underlying the effects of prenatal psychosocial stress on child outcomes: beyond the HPA axis. European child & adolescent psychiatry 23: 943-956, 2014.

10. Benjamin EJ, Virani SS, Callaway CW, Chamberlain AM, Chang AR, Cheng S, Chiuve SE, Cushman M, Delling FN, and Deo R. Heart disease and stroke statistics-2018 update: a report from the American Heart Association. Circulation 137: e67, 2018.

11. Bennett IM, Schott W, Krutikova S, and Behrman JR. Maternal mental health, and child growth and development, in four low-income and middle-income countries. J Epidemiol Community Health 70: 168-173, 2016.

12. Bertram CE & Hanson MA. Animal models and programming of the metabolic syndrome: Type 2 diabetes. *British medical bulletin*, *60*(1), 103-121. 2001

13. Bian X, Seidler F, and Slotkin T. Promotional role for glucocorticoids in the development of intracellular signalling: enhanced cardiac and renal adenylate cyclase reactivity to beta-adrenergic and non-adrenergic stimuli after low-dose fetal dexamethasone exposure. Journal of developmental physiology 17: 289-297, 1992.

14. Blood AB, Zhao Y, Long W, Zhang L, and Longo LD. L-type Ca2+ channels in fetal and adult ovine cerebral arteries. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 282: R131-R138, 2002.

15. Brem AS, Bina RB, Mehta S, and Marshall J. Glucocorticoids inhibit the expression of calcium-dependent potassium channels in vascular smooth muscle. Molecular genetics and metabolism 67: 53-57, 1999.

16. Buckley NM. Maturation of circulatory system in three mammalian models of human development. Comparative Biochemistry and Physiology Part A: Physiology 83: 1-7, 1986.

17. Celsi G, Kistner A, Aizman R, EklÖF A-C, Ceccatelli S, De Santiago A, and Jacobson SH. Prenatal dexamethasone causes oligonephronia, sodium retention, and higher blood pressure in the offspring. Pediatr Res 44: 317, 1998.

18. Cohlen BJ, Stigter RH, Derks JB, Mulder EJH, and Visser GHA. Absence of significant hemodynamic changes in the fetus following maternal betamethasone administration. Ultrasound Obst Gyn 8: 252-255, 1996.

19. Cowley Jr AW. Long-term control of arterial blood pressure. Physiological reviews 72: 231-300, 1992.

20. Dampney R, Coleman M, Fontes M, Hirooka Y, Horiuchi J, Li YW, Polson J, Potts P, and Tagawa T. Central mechanisms underlying short-and long-term regulation of the cardiovascular system. Clinical and experimental pharmacology and physiology 29: 261-268, 2002.

21. de Catanzaro D. and Macniven E. Psychogenic pregnancy disruptions in mammals. *Neuroscience & Biobehavioral Reviews*, *16*(1), pp.43-53. 1992

22. Deren O, Karaer C, Onderoglu L, Yigit N, Durukan T, and Bahado-Singh RO. The effect of steroids on the biophysical profile and Doppler indices of umbilical and middle cerebral arteries in healthy preterm fetuses. Eur J Obstet Gyn R B 99: 72-76, 2001.

23. Derks JB, Giussani DA, Jenkins SL, Wentworth RA, Visser G, Padbury J, and Nathanielsz P. A comparative study of cardiovascular, endocrine and behavioural effects of betamethasone and dexamethasone administration to fetal sheep. The Journal of Physiology 499: 217-226, 1997.

24. Docherty CC, Kalmar-Nagy J, Engelen M, and Nathanielsz PW. Development of fetal vascular responses to endothelin-1 and acetylcholine in the sheep. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 280: R554-R562, 2001.

25. Dodic M, May C, Wintour E, and Coghlan J. An early prenatal exposure to excess glucocorticoid leads to hypertensive offspring in sheep. Clin Sci 94: 149-155, 1998.

26. Dodic M, Samuel C, Moritz K, Wintour EM, Morgan J, Grigg L, and Wong J. Impaired cardiac functional reserve and left ventricular hypertrophy in adult sheep after prenatal dexamethasone exposure. Circulation research 89: 623-629, 2001.

27. Dreiling M, Schiffner R, Bischoff S, Rupprecht S, Kroegel N, Schubert H, Witte OW, Schwab M, and Rakers F. Impact of chronic maternal stress during early gestation on maternal–fetal stress transfer and fetal stress sensitivity in sheep. Stress 21: 1-10, 2018.

28. Falah-Hassani K, Shiri R, and Dennis C-L. The prevalence of antenatal and postnatal co-morbid anxiety and depression: a meta-analysis. Psychological medicine 47: 2041-2053, 2017.

29. Faraji J, Soltanpour N, Ambeskovic M, Zucchi FC, Beaumier P, Kovalchuk I and Metz GA, Evidence for ancestral programming of resilience in a two-hit stress model. *Frontiers in behavioral neuroscience*, *11*, p.89. 2017

30. Fowden A and Forhead A. Endocrine mechanisms of intrauterine programming. Reproduction 127: 515-526, 2004.

31. Fowden AL and Forhead AJ. Glucocorticoids as regulatory signals during intrauterine development. Experimental physiology 100: 1477-1487, 2015.

32. Fowden AL, Li J, and Forhead AJ. Glucocorticoids and the preparation for life after birth: are there long-term consequences of the life insurance? Proceedings of the Nutrition Society 57: 113-122, 1998.

33. Frasch M, Müller T, Wicher C, Weiss C, Löhle M, Schwab K, Schubert H, Nathanielsz P, Witte O, and Schwab M. Fetal body weight and the development of the control of the cardiovascular system in fetal sheep. The Journal of physiology 579: 893-907, 2007.

34. Goyal R, Mittal A, Chu N, Shi L, Zhang L, and Longo LD. Maturation and the role of PKC-mediated contractility in ovine cerebral arteries. American Journal of Physiology-Heart and Circulatory Physiology 297: H2242-H2252, 2009.

35. Grier DG and Halliday HL. Effects of glucocorticoids on fetal and neonatal lung development. Treatments in respiratory medicine 3: 295-306, 2004.

36. Hai C-M, Sadowska G, Francois L, and Stonestreet BS. Maternal dexamethasone treatment alters myosin isoform expression and contractile dynamics in fetal arteries.

American Journal of Physiology-Heart and Circulatory Physiology 283: H1743-H1749, 2002.

37. Hanson M. The control of heatrate and blood pressure in the fetus: theoretical considerations. Fetus and Neonate: physiology and clinical appricatons 1: 1-22, 1993.

38. Hinchliffe S, Lynch M, Sargent P, Howard C, and Van Velzen D. The effect of intrauterine growth retardation on the development of renal nephrons. BJOG: An International Journal of Obstetrics & Gynaecology 99: 296-301, 1992.

39. Holsboer F. Stress, hypercortisolism and corticosteroid receptors in depression: implicatons for therapy. J Affect Disorders 62: 77-91, 2001.

40. Igosheva N, Klimova O. Anishchenko T, Glover V. Prenatal stress alters cardiovascular responses in adult rats. J Physiol-London 557, 273-285. 2004.

41. Jones CT, Robinson RO. Plasma catecholamines in fetal and adult sheep. J Physiol. 248:15-33. 1975

42. Kähler C, Schleussner E, Möller A and Seewald HJ. Doppler measurements in fetoplacental vessels after maternal betamethasone administration. Fetal Diagn Ther 19: 52-57, 2004.

43. Kapoor A & Matthews SG. Short periods of prenatal stress affect growth, behaviour and hypothalamo-pituitary-adrenal axis activity in male guinea pig offspring. J Physiol-London 566, 967-977. 2005.

44. King S, Dancause K, Turcotte-Tremblay AM, Veru F, and Laplante DP. Using natural disasters to study the effects of prenatal maternal stress on child health and development. Birth Defects Research Part C: Embryo Today: Reviews 96: 273-288, 2012.

45. Kutzler MA, Ruane EK, Coksaygan T, Vincent SE, and Nathanielsz PW. Effects of three courses of maternally administered dexamethasone at 0.7, 0.75, and 0.8 of gestation on prenatal and postnatal growth in sheep. Pediatrics 113: 313-319, 2004.

46. Lee J-H, Zhang J, Massmann G, and Figueroa J. Antenatal betamethasone increases vascular reactivity to endothelin-1 by upregulation of CD38/cADPR signaling. J Dev Orig Hlth Dis 5: 56-62, 2014.

47. Lewko B, Wendt U, Stepinski J, and Angielski S. Dexamethasone sensitizes soluble guanylate cyclase in the rat renal glomeruli. Biochemical and biophysical research communications 197: 826-832, 1993.

48. Liggins G. Howie. A controlled trail of antepartum glucocorticoid treatment for prevention of RDS in premature infants. Pediatrics 54: 724, 1974.

49. Lohle M, Muller T, Wicher C, Roedel M, Schubert H, Witte OW, Nathanielsz PW, and Schwab M. Betamethasone effects on fetal sheep cerebral blood flow are not dependent on maturation of cerebrovascular system and pituitary-adrenal axis. J Physiol-London 564: 575-588, 2005.

50. Long W, Zhang L, and Longo LD. Cerebral artery sarcoplasmic reticulum Ca2+ stores and contractility: changes with development. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 279: R860-R873, 2000.

51. Lorijn R and Longo L. Norepinephrine elevation in the fetal lamb: oxygen consumption and cardiac output. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 239: R115-R122, 1980.

52. Mackenzie HS and Brenner BM. Fewer nephrons at birth: a missing link in the etiology of essential hypertension? American journal of kidney diseases 26: 91-98, 1995.

53. Malhotra A, Ditchfield M, Fahey M, Castillo-Melendez M, Allison B, Polglase G, Wallace E, Hodges R, Jenkin G, and Miller S. Detection and assessment of brain injury in the growth restricted fetus and neonate. Pediatr Res, 2017.

54. Martyn C, Barker D, Jespersen S, Greenwald S, Osmond C, and Berry C. Growth in utero, adult blood pressure, and arterial compliance. Heart 73: 116-121, 1995.

55. Matuszek MA, Gibson KJ, Lumbers ER, and Simonetta G. IMPACT OF CORTISOL ON α-ACTIN CONTENT IN VASCULAR SMOOTH MUSCLE CELLS OF FETAL SHEEP. Clinical and experimental pharmacology and physiology 33: 197-203, 2006.

56. McMillen IC and Robinson JS. Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. Physiological reviews 85: 571-633, 2005.

57. Molnar J, Nijland MJ, Howe DC, and Nathanielsz PW. Evidence for microvascular dysfunction after prenatal dexamethasone at 0.7, 0.75, and 0.8 gestation in sheep. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 283: R561-R567, 2002.

58. Moritz KM, Boon WM, and Wintour EM. Glucocorticoid programming of adult disease. Cell and tissue research 322: 81-88, 2005.

59. Moritz KM, Johnson K, Douglas-Denton R, Wintour EM, & Dodic M. Maternal glucocorticoid treatment programs alterations in the renin-angiotensin system of the ovine fetal kidney. *Endocrinology*, *143*(11), 4455-4463. 2002

60. Moss TJ, Sloboda DM, Gurrin LC, Harding R, Challis JR & Newnham JP. Programming effects in sheep of prenatal growth restriction and glucocorticoid exposure. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *281*(3), R960-R970. 2001

61. Nuyt AM. Mechanisms underlying developmental programming of elevated blood pressure and vascular dysfunction: evidence from human studies and experimental animal models. Clin Sci 114: 1-17, 2008.

62. Mulvany MJ & Aalkjær C. Structure and function of small arteries. *Physiological reviews*, *70*(4), 921-961. 1990

63. Mulvany MJ and Halpern W. Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. *Circulation research* 41: 19-26, 1977.

64. Pariante CM and Miller AH. Glucocorticoid receptors in major depression: relevance to pathophysiology and treatment. Biol Psychiat 49: 391-404, 2001.

65. Peers A, Campbell DJ, Wintour EM, and Dodic M. The peripheral renin–angiotensin system is not involved in the hypertension of sheep exposed to prenatal dexamethasone. Clinical and Experimental Pharmacology and Physiology 28: 306-311, 2001.

66. Plagemann A. Fetale Programmierung und funktioneile Teratologie. In: Molekularmedizinische Grundlagen von fetalen und neonatalen Erkrankungen: Springer, 2005, p. 325-344.

67. Plana-Ripoll O, Liu XQ, Momen NC, Parner E, Olsen J, and Li J. Prenatal exposure to maternal stress following bereavement and cardiovascular disease: A nationwide population-based and sibling-matched cohort study. Eur J Prev Cardiol 23: 1018-1028, 2016.

68. Pulgar VM and Figueroa JP. Antenatal betamethasone administration has a dual effect on adult sheep vascular reactivity. Pediatr Res 60: 705, 2006.

69. Quaedackers JS, Roelfsema V, Fraser M, Gunn AJ, and Bennet L. Cardiovascular and endocrine effects of a single course of maternal dexamethasone treatment in preterm fetal sheep. BJOG: An International Journal of Obstetrics & Gynaecology 112: 182-191, 2005.

70. Rakers F, Bischoff S, Schiffner R, Haase M, Rupprecht S, Kiehntopf M, Kühn-Velten WN, Schubert H, Witte OW, and Nijland MJ. Role of catecholamines in maternal-fetal stress transfer in sheep. Am J Obstet Gynecol 213: 684. e681-684. e689, 2015.

71. Rakers F, Frauendorf V, Rupprecht S, Schiffner R, Bischoff SJ, Kiehntopf M, Reinhold P, Witte OW, Schubert H, and Schwab M. Effects of early-and late-gestational maternal stress and synthetic glucocorticoid on development of the fetal hypothalamus–pituitary–adrenal axis in sheep. Stress 16: 122-129, 2013.

72. Rakers F, Rupprecht S, Dreiling M, Bergmeier C, Witte OW, and Schwab M. Transfer of maternal psychosocial stress to the fetus. Neuroscience & Biobehavioral Reviews, 2017.

73. Reinisch JM, Simon NG, Karow WG, and Gandelman R. Prenatal exposure to prednisone in humans and animals retards intrauterine growth. Science 202: 436-438, 1978.

74. Robillard JE and Nakamura KT. Neurohormonal regulation of renal function during development. American Journal of Physiology-Renal Physiology 254: F771-F779, 1988.

75. Roghair RD, Lamb FS, Bedell KA, Smith OM, Scholz TD, and Segar JL. Late-gestation betamethasone enhances coronary artery responsiveness to angiotensin II in fetal sheep. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 286: R80-R88, 2004.

76. Roghair RD, Segar JL, Sharma RV, Zimmerman MC, Jagadeesha D, Segar EM, Scholz TD, and Lamb FS. Newborn lamb coronary artery reactivity is programmed by early gestation dexamethasone before the onset of systemic hypertension. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 289: R1169-R1176, 2005.

77. Roos A, Geerts L, Koen N, Faure SC, Vythilingum B, and Stein DJ. Psychosocial predictors of fetoplacental blood flow dunng pregnancy. Compr Psychiat 57: 125-131, 2015.

78. Rose JC, Kute TE, and Winkler L. Glucocorticoid receptors in sheep brain tissues during development. American Journal of Physiology-Endocrinology and Metabolism 249: E345-E349, 1985.

79. Rotmensch S, Liberati M, Vishne TH, Celentano C, Ben-Rafael Z, and Bellati U. The effect of betamethasone and dexamethasone on fetal heart rate patterns and biophysical activities - A prospective randomized trial. Acta Obstet Gyn Scan 78: 493-500, 1999.

80. Roussel S, Hemsworth PH, Boissy A, Duvaux-Ponter C. Effects of repeated stress during pregnancy in ewes on the behavioural and physiological responses to stressful events and birth weight of their offspring. Appl. Anim. Behav. Sci. 85,259–276- 2004

81. Rupprecht S, Frauendorf V, Rakers F, Schubert H, Witte OW, and Schwab M. Isolation of the Pregnant Ewe Is a Valid Stress Model. REPRODUCTIVE SCIENCES. SAGE PUBLICATIONS INC 2455 TELLER RD, THOUSAND OAKS, CA 91320 USA, 2009, p. 337A-337A.

82. Schleußner E. Fetale Programmierung. In: Die Geburtshilfe: Springer, 2011, p. 617-632.

83. Schwab M, Roedel M, Anwar MA, Müller T, Schubert H, Buchwalder LF, Walter B, and Nathanielsz PW. Effects of betamethasone administration to the fetal sheep in late gestation on fetal cerebral blood flow. The Journal of physiology 528: 619-632, 2000.

84. Seckl JR. Glucocorticoid programming of the fetus; adult phenotypes and molecular mechanisms. Molecular and cellular endocrinology 185: 61-71, 2001.

85. Seckl JR and Meaney MJ. Glucocorticoid programming. Annals of the New York Academy of Sciences, 1032(1), pp.63-84. 2004

86. Segar JL, Roghair RD, Segar EM, Bailey MC, Scholz TD, and Lamb FS. Early gestation dexamethasone alters baroreflex and vascular responses in newborn lambs before hypertension. *Am J Physiol-Reg I* 291: R481-R488, 2006.

87. Slotkin T, Lau C, McCook E, Lappi S, and Seidler F. Glucocorticoids enhance intracellular signaling via adenylate cyclase at three distinct loci in the fetus: a mechanism for heterologous teratogenic sensitization? Toxicology and applied pharmacology 127: 64-75, 1994.

88. Smith SM and Vale WW. The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. Dialogues in clinical neuroscience 8: 383, 2006.

89. Stein HM, Oyama K, Martinez A, Chappell BA, Buhl E, Blount L, and Padbury JF. Effects of corticosteroids in preterm sheep on adaptation and sympathoadrenal mechanisms at birth. American Journal of Physiology-Endocrinology And Metabolism 264: E763-E769, 1993.

90. Tangalakis K, Lumbers E, Moritz K, Towstoless M, and Wintour E. Effect of cortisol on blood pressure and vascular reactivity in the ovine fetus. Experimental Physiology: Translation and Integration 77: 709-717, 1992.

91. Teixeira JMA, Fisk NM, and Glover V. Association between maternal anxiety in pregnancy and increased uterine artery resistance index: cohort based study. Brit Med J 318: 153-157, 1999.

92. Ullian ME. The role of corticosteroids in the regulation of vascular tone. Cardiovascular research 41: 55-64, 1999.

93. Ulrich-Lai YM and Herman JP. Neural regulation of endocrine and autonomic stress responses. Nature reviews neuroscience 10: 397, 2009.

94. Unno N, Wong CH, Jenkins SL, Wentworth RA, Ding X-Y, Li C, Robertson SS, Smotherman WP, and Nathanielsz PW. Blood pressure and heart rate in the ovine fetus: ontogenic changes and effects of fetal adrenalectomy. American Journal of Physiology-Heart and Circulatory Physiology 276: H248-H256, 1999.

95. Vackova Z, Vagnerova K, Libra A, Miksik I, Pacha J, and Staud F. Dexamethasone and betamethasone administration during pregnancy affects expression and function of 11β-hydroxysteroid dehydrogenase type 2 in the rat placenta. Reprod Toxicol 28: 46-51, 2009.

96. Van den Bergh BR, Van Calster B, Smits T, Van Huffel S, and Lagae L. Antenatal maternal anxiety is related to HPA-axis dysregulation and self-reported depressive symptoms in adolescence: a prospective study on the fetal origins of depressed mood. Neuropsychopharmacol 33: 536, 2008.

97. van Dijk AE, Dawe K, Deanfield J, Stronks K, Gemke RJBJ, Vrijkotte TGM, and Lawlor DA. The association of maternal prenatal psychosocial stress with vascular function in the child at age 10-11 years: findings from the Avon longitudinal study of parents and children. Eur J Prev Cardiol 21: 1097-1108, 2014.

98. van Dijk AE, van Eijsden M, Stronks K, Gemke RJBJ, and Vrijkotte TGM. The Association between Prenatal Psychosocial Stress and Blood Pressure in the Child at Age 5-7 Years. Plos One 7, 2012.

99. Vythilingum B, Geerts L, Fincham D, Roos A, Faure S, Jonkers J, and Stein DJ. Association between antenatal distress and uterine artery pulsatility index. Arch Women Ment Hlth 13: 359-364, 2010.

100. Wadhwa PD, Sandman CA, Porto M, Dunkel-Schetter C, and Garite TJ. The association between prenatal stress and infant birth weight and gestational age at birth: a prospective investigation. Am J Obstet Gynecol 169: 858-865, 1993.

101. Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, Dymov S, Szyf M, and Meaney MJ. Epigenetic programming by maternal behavior. Nature neuroscience 7: 847, 2004.

102. Wei W, Graeff R, and Yue J. Roles and mechanisms of the CD38/cyclic adenosine diphosphate ribose/Ca2+ signaling pathway. World journal of biological chemistry 5: 58, 2014.

103. Wintour E, Johnson K, Koukoulas I, Moritz K, Tersteeg M, and Dodic M. Programming the cardiovascular system, kidney and the brain—a review. Placenta 24: S65-S71, 2003.

104. WINTOUR EM and MORITZ KM. Comparative aspects of fetal renal development. Equine Veterinary Journal 29: 51-58, 1997.

105. Yang K, Jones S, and Challis J. Changes in glucocorticoid receptor number in the hypothalamus and pituitary of the sheep fetus with gestational age and after adrenocorticotropin treatment. Endocrinology 126: 11-17, 1990.

Anhang

A. Die funktionelle Entwicklung renaler Interlobulararteriolen vom fetalen Schaf

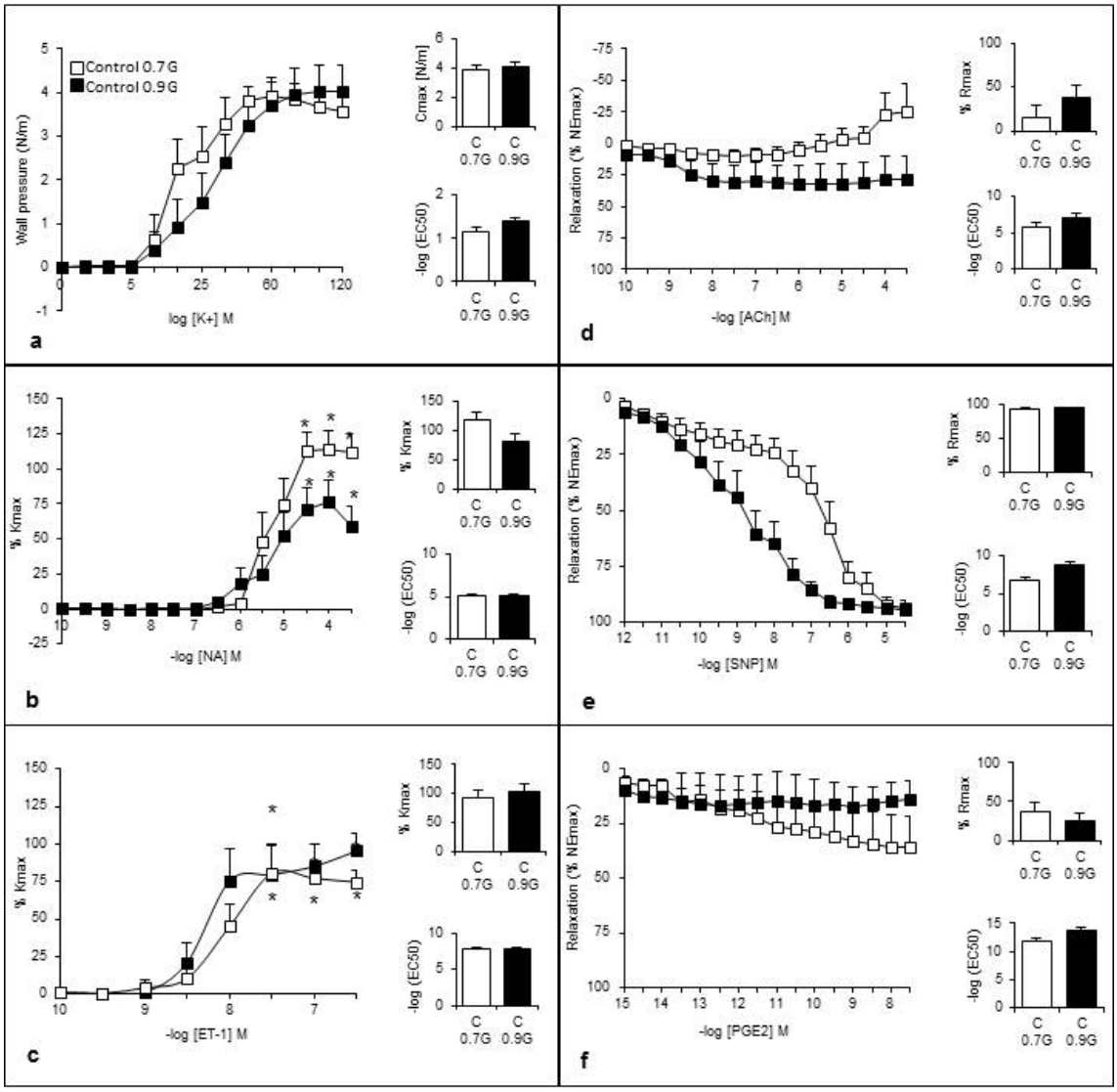


Abbildung 4: Reaktion der renalen Intertobulararteriolen auf Vasokonstriktoren und Vasodilatoren im fetalen Schaf.

Werte sind Mittelwerte ± SEM für die Konzentrations-Respons-Kurven, die maximal Konstriktion (C_{max} oder $\%K_{max}$), und die Sensitivität (EC_{50} oder $-\log(EC_{50})$) zu Kalium (K^+) (**a**), Noradrenalin (NA) (**b**), Endothelin-1 (ET-1) (**c**), als auch die maximale Relaxation ($\%R_{max}$) und Sensitivität ($-\log(EC_{50})$) zu Acetylcholin (ACh) (**d**), Sodium nitroprussid (SNP) (**e**) und Prostaglandin-E2 (PGE_2) (**f**). Gruppen sind 0.7 der Gestation (0.7, weiße Quadrate) und 0.9 der Gestation (0.9, schwarze Quadrate). Signifikante Unterschiede zwischen den Gruppen ($p \leq 0.05$) sind: ***(**two-way ANOVA for repeated measurements+ post hoc Tukey HSD correction)

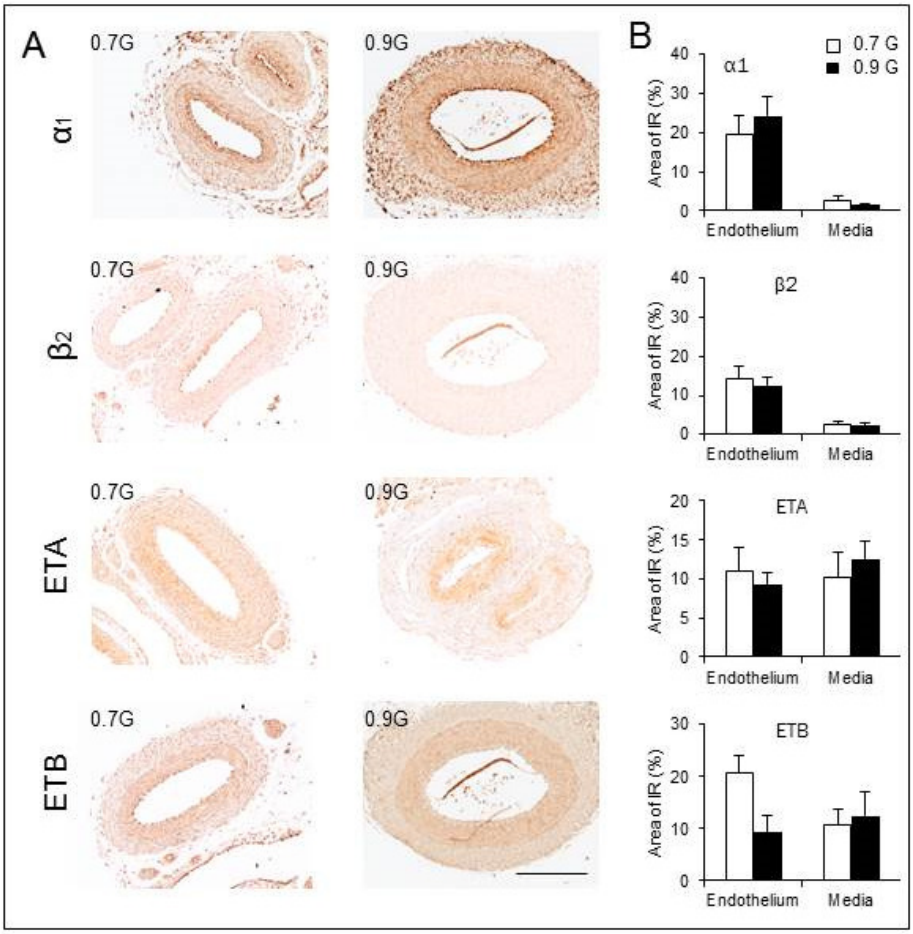


Abbildung 5: Die Expression der an der Vasokonstriktion beteiligten Rezeptoren in fetalen renalen Interlobulararteriolen

A, repräsentative mikroskopische Aufnahmen der immunhistochemischen Färbung des Adrenorezeptor α_{1A} (α_{1A}), Adrenorezeptor β_2 (β_2), Endothelin-1 Rezeptor type A (ET_A), Endothelin-1 Rezeptor type B (ET_B) bei 0.7 (0.7 G) und 0.9 (0.9 G) der ovinen Gestation. **B,** Bildanalysen der spezifischen immunhistochemischen Färbung des Endothels und der Media. Media bei 0.7 (0.7 G) und 0.9 (0.9 G) der ovinen Gestation. Die Balken stellen das Verhältnis der spezifisch gefärbten Fläche zur Media dar. Werte sind Mittelwerte ± SEM, Maßstabsbalken 100 µm. Die Gruppen sind 0.7 der Gestation (weiße Balken) und 0.9 der Gestation (schwarze Balken).

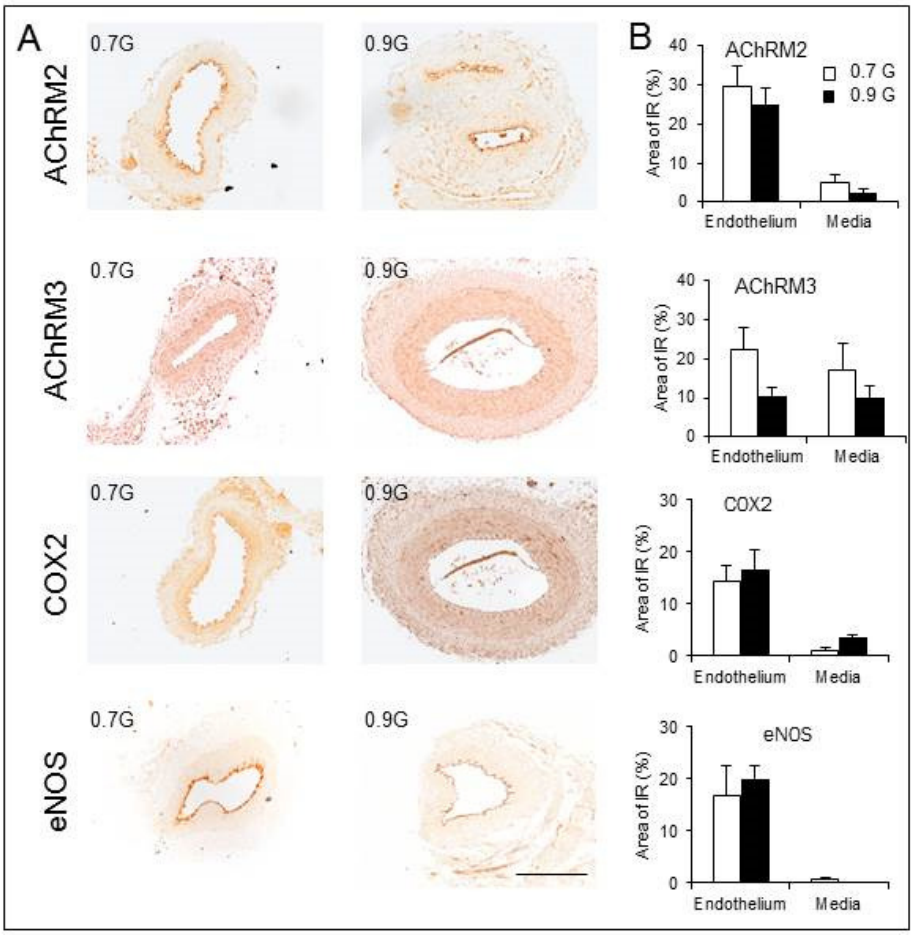


Abbildung 6: Die Expression der an der Vasodilatation beteiligten Rezeptoren und Enzyme in fetalen renalen Interlobulararteriolen.

A, repräsentative mikroskopische Aufnahmen der immunhistochemischen Färbung der muskarinischer Acetylcholinerezeptor M2 und M3 (AChRM2, AChRM3), der Cyklooxygenase-2 (COX-2), der endothelialen NO-Synthase (eNOS) bei 0.7 (0.7 G) und 0.9 (0.9 G) der ovinen Gestation. **B**, Bildanalysen der spezifischen immunhistochemischen Färbung des Endothels und der Media. Media bei 0.7 (0.7 G) und 0.9 (0.9 G) der ovinen Gestation. Die Balken stellen das Verhältnis der spezifisch ge-färbten Fläche zur Media dar. Werte sind Mittelwerte ± SEM, Maßstabsbalken 200 µm. Die Gruppen sind 0.7 der Gestation (weiße Balken) und 0.9 der Gestation (schwarze Balken).

Tabelle 2: Fetale renale Rezeptorexpression mittels des Verhältnisses der positive gefärbten Fläche im Endothel und der Media bei 0.7 und 0.9 der ovinen Gestation

Marker	Ratio of area for IR								Changes in E/M ratio between 0.7 and 0.9 gestation
	0.7 gestation				0.9 gestation				
	E	M	E/M ratio, %	p	E	M	E/M ratio, %	p	p
AChRM2	.29±.05*	.05±.02	9.26±2.59	.001	.25±.04*	.023±.009	26.00±10.95	.004	.75
AChRM3	.22±.06	0.17±0.07	2.76±1.28	.232	0.10±0.02	0.10±0.03	1.27±0.24	.928	.59
α_1	.19±.05*	0.025±0.014	97.13±45.43	.009	.22±.04*	.013±.004	28.61±12.42	.007	.77
β_2	.14±0.03*	.026±.016	7.78±3.63	.015	.10±.02*	.019±.003	5.27±0.71	.006	1.00
COX-2	.14±.03*	.01±0.005	36.13±16.04	.002	0.17±0.02*	.04±.01	5.83±1.35	.001	.54
eNOS	.17±.06*	.006±.005	691.26±331.32	.021	0.20±0.03*	.0005±.0002	739.94±266.41	.001	1.00
ET_A	.11±.03	.1±.03	1.23±0.26	.649	0.09±0.02	.12±.02	.80±.12	.141	.75
ET_B	.21±.04*	.11±.04	4.43±1.95	.010	.12±0.03	.08±.05	9.02±5.01	.225	.79

Werte sind Mittelwerte ± SEM, Endothel (E), Media (M), muskarinischer Acetylcholinerezeptor M2 (AChRM2), muskarinischer Acetylcholinerezeptor M3 (AChRM3), Adrenorezeptor α_{1A} (α_{1A}), Adrenorezeptor α_{2A} (α_{2A}), Adrenorezeptor β_2 (β_2), Cycloogenase-2 (COX-2), endotheliale NO-synthase (eNOS), Endothelin-1 Rezeptor type A (ET_A), Endothelin-1 Rezeptor type B (ET_B), Prostaglandin-E2 Rezeptor EP2 (EP2), Prostaglandin-E_2 Rezeptor EP4 (EP4).

B. Die strukturelle Entwicklung renaler Interlobulararteriolen im fetalen Schaf

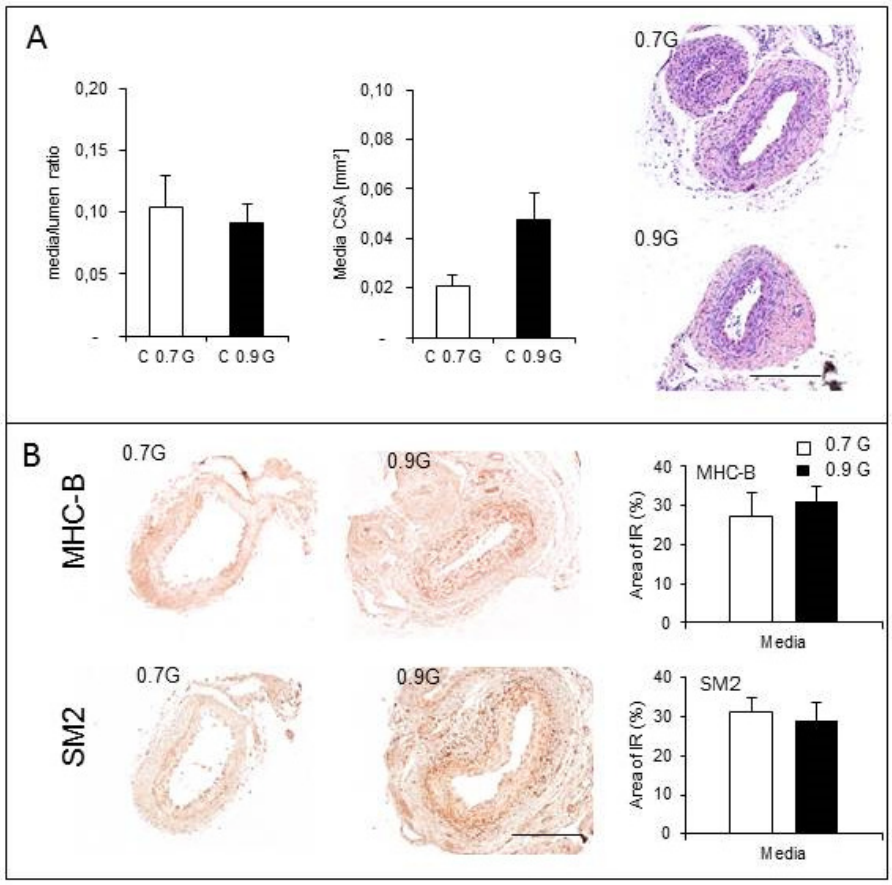


Abbildung 7: Der Aufbau der Gefäßwand fetaler renalen Interlobulararteriolen.

A, Die Balken stellen Media/lumen Verhältnis und die *media cross sectional area* (CSA) grafisch dar. Repräsentative mikroskopische Aufnahmen der HE Färbung der fetalen renalen Interlobulararteriolen bei 0.7 (0.7 G) und 0.9 (0.9 G) der ovinen Gestation. **B,** repräsentative mikroskopische Aufnahmen der Färbung von fetaler MHC-B und adulter SM2- *myosin heavy chain* Isoformen der fetalen renalen Interlobulararteriolen bei 0.7 und 0.9 der ovinen Gestation (0.7 G and 0.9 G). sowie Bildanalyse der spezifischen immunohistochemischen Färbung der Media bei 0.7 (0.7 G) und 0.9 (0.9 G) der ovinen Gestation.. Die Balken stellen das Verhältnis der spezifisch gefärbten Fläche zur Media dar. Die Werte sind Mittelwerte ± SEM, Maßstabsbalken 200 µm. Die Gruppen sind 0.7 der Gestation (weiße Balken) und 0.9 der Gestation (schwarze Balken).

Angaben zum Eigenanteil

Fetal sheep mesenteric resistance arteries: functional and structural maturation.

Muller, J.J., Schwab, M., Rosenfeld, C.R., Antonow-Schlorke, I., Nathanielsz, P.W., Rakers, F., Schubert, H., Witte, O.W., Rupprecht, S. 259-271, Fetal Sheep Mesenteric Resistance Arteries: Functional and Structural Maturation. J Vasc Res 54. 2017

Planung und Durchführung der experimentellen Arbeiten: MS, SR, JJM

Methodenetablierung
a) fetales Schaf als Tiermodell: MS, HS, FR, SR
b) wire myography: SR,
c) Immunhistochemie: IA, CRR, JJM

Datenerfassung und Auswertung: SR, JJM

statistische Berechnungen: SR, JJM

Erstellung des Manuskripts: SR, PWN, OWW, JJM

Erstellung der Abbildungen: JJM

Impact of chronic maternal psychosocial stress on the development of fetal blood pressure regulating arteries in sheep.

Julia J. Müller, Matthias Schwab, Florian Rakers, Charles R. Rosenfeld, Iwa Antonow-Schlorke, Peter W. Nathanielsz, Harald Schubert, Otto W. Witte, Sven Rupprecht. Manuskript Einreichung vorgesehen im Journal *Stress*

Planung und Durchführung der experimentellen Arbeiten: MS, SR, JJM

Methodenetablierung
a) fetales Schaf als Tiermodell: MS, HS, FR, SR
b) wire myography: SR
c) Immunhistochemie: IA, CRR, JJM

Datenerfassung und Auswertung: SR, JJM

statistische Berechnungen: SR, JJM

Erstellung des Manuskripts: SR, PWN, OWW, JJM

Erstellung der Abbildungen: JJM

Cardiovascular effects of prenatal stress – Are there implications for cerebrovascular, cognitive and mental health outcome?

Muller, J.J., Antonow-Schlorke I., Kroegel, N., Rupprecht, S., Rakers, F., Witte, O.W., Schwab, M.

Veröffentlicht im Journal of Neuroscience and Biobehavioral Reviews 2018. S.

Einleitung: IA, JJM, MS

Effekte auf die Entwicklung im Menschen: IA

Effekte auf die Entwicklung in Tiermodellen: JJM

Erklärung der Effekte: FR, MS, JJM,

Zusammenfassung, Ausblick: IA, JJM, MS

Lektorat: MS, OWW, NK

(Co) authors:

CRR: Rosenfeld, C.R.,
FR: Rakers, F.,
HS: Schubert, H.,
IA: Antonow-Schlorke, I.,
JJM: Muller, J.J.,
MS: Schwab, M.,
NK: Kroegel, N.,
OWW: Witte, O.W.,
PWN: Nathanielsz, P.W.,
SR: Rupprecht, Sven

Ehrenwörtliche Erklärung

Name:	Müller	Vorname:	Julia Josephine
Matrikel-Nr.:	106037	Studiengang:	Molecular Medicine

Hiermit erkläre ich, dass mir die Promotionsordnung der Medizinischen Fakultät der Friedrich-Schiller-Universität Jena bekannt ist,

ich die Dissertation selbst angefertigt habe und alle von mir benutzten Hilfsmittel, persönlichen Mitteilungen und Quellen in meiner Arbeit angegeben sind,

mich folgende Personen bei der Auswahl und Auswertung des Materials sowie bei der Erstellung des Manuskripts unterstützt haben:

Prof. Dr. med. Matthias Schwab und Dr. med. Sven Rupprecht, Klinik für Neurologie des Universitätsklinikum Jena,

bei der Erarbeitung der Zielstellung und Diskussion der Ergebnisse,

Dipl.-Biol. Iwa Antonow-Schlorke, Ina Ingrisch, MTA, Claudia Sommer, MTA, Klinik für Neurologie des Universitätsklinikum Jena, bei der histologischen und immunhistochemischen Aufarbeitung,

die Hilfe eines Promotionsberaters nicht in Anspruch genommen wurde und dass Dritte weder unmittelbar noch mittelbar geldwerte Leistungen von mir für Arbeiten erhalten haben, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen, dass ich die Dissertation noch nicht als Prüfungsarbeit für eine staatliche oder andere wissenschaftliche Prüfung eingereicht habe und

dass ich die gleiche, eine in wesentlichen Teilen ähnliche oder eine andere Abhandlung nicht bei einer anderen Hochschule als Dissertation eingereicht habe.

_____ _____

Ort, Datum Unterschrift

Danksagung

Hiermit möchte ich meinen besonderen Dank nachstehenden Personen entgegenbringen, welche mir durch Ihre Unterstützung die Anfertigung dieser Promotionsschrift erst ermöglicht haben:

Mein Dank gilt Herrn Prof. Dr. med. Matthias Schwab, Leiter der Arbeitsgruppe fetale Programmierung für die Vergabe der Thematik, die Begutachtung dieser Arbeit, der anspruchsvollen Diskussionen, die mir einen kritischen Zugang zu dieser Thematik eröffneten.

Von ganzem Herzen danke ich Herrn Dr. med. Sven Rupprecht für die kontinuierliche Betreuung meiner Promotion, die mehrfache Durchsicht dieser Abhandlung und der darin enthaltenen Manuskripte sowie die Unterstützung und die Zusammenarbeit während der Experimente waren ein wesentlicher Bestandteil zur Erstellung dieser Arbeit. Ohne seine experimentelle Erfahrung in der Methode der *small vessel wire myography* und die Unterstützung von Claudia Sommer, wäre die Durchführung der Experimente am Schafgewebe nicht möglich gewesen.

Zudem möchte ich mich bei Frau Dipl.-Biol. Iwa Antonow-Schlorke bedanken für die Einführung in das immunhistochemische Arbeiten und die gemeinsame Arbeit am Review.

Des Weiteren bedanke ich mich bei allen Mitgliedern der Arbeitsgruppen „experimentelle Neurologie“ und „fetale Programmierung", sowie den Mitarbeitern des Instituts für Versuchstierkunde der FSU Jena für die technische Unterstützung und das freundliche Arbeitsklima. Ein besonderer Dank geht dabei an Claudia Sommer und Ina Ingrisch für ihre stetige praktische Hilfe und Ihre freundliche Unterstützung in allen Fragestellungen.

Meine ehemalige Kommilitonin und gute Freundin Franziska Eberl möchte ich herzlich danken, für ihre freundliche Hilfe und die kritischen Anmerkungen. Sie hat zur Erstellung der Promotionsschrift wesentlich beigetragen.

Tief verbunden und dankbar bin ich meinen Eltern Birgit und Peter Müller sowie meiner Schwester Katrin Müller. Ohne Ihre liebevolle Unterstützung und die fortwährende Motivation, wäre dieser Abschluss vielleicht nicht möglich gewesen.